FUNGAL DISEASES OF ROOT VEGETABLES

A Thesis

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BY

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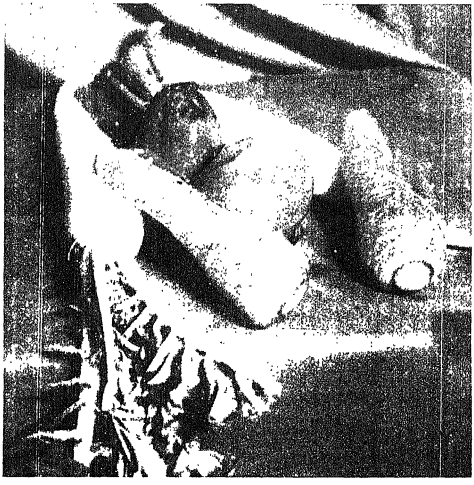

Root vegetables under investigation

CERTIFICATE

Certified that the thesis embodies results of original research work and study carried out under my supervision by Mr. Manoj Kumar Mishra M.Sc. (Ag.) Botany.

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PREFACE

The thesis embodies research work on **"Fungal Diseases of Root Vegetables"**. The work carried out by me from January 2000 to March 2002 in the Someshwer Nath Bhargva Agricultural Botany Laboratory, Garden and Farm of Botany Department, University of Allahabad.

The thesis embodies researches with studies on some mycoflora isolated from root vegetables. The thesis contains work on Isolation and Pathogological studies, Effect of Storage Conditions, Environmental effect on some mycoflora with their respective vegetable seeds, infected plant parts, field soils and their control studies.

The thesis is compiled into Eleven Chapters. First chapter deals with Introduction followed by Material and Methods (Chapter - 2) Isolation and Pathological Studies are discussed in Chapter - 3 and Chapter - 4 Environmental Studies and Storage Studies are given in Chapter - 5 and Chapter - 6 respectively. Chapter - 7 deals with Survival Studies. Control Studies are discussed in Chapter -8.

A detailed Discussion on the basis of the present investigation and the Summary are given in Chapter - 9 and Chapter - 10. The bibliography containing the references cited in the (Chapter - 11). Abstract of the present work is also submitted along with the thesis separately.

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CONTENTS

CHAPTER - 1

INTRODUCTION

INTRODUCTION

After independence one of the task of the National Government of India was to develop a viable and productive agricultural economy leading to self-sufficiency in our food requirement. Several steps and planned efforts have been made to give effect to this objective. The country is now almost self-sufficient in matter of food grains. However self-sufficiency in the true sense can be achieved only when each individual in the country is assumed of a balanced diet. Fruits and vegetables are the only natural sources of protective food, as they supply nutrients, vitamins and minerals. In a country where the population is predominantly vegetarian this can only be achieved by increasing the production and consumption of vegetables. In India, the consumption of vegetable is comparatively five times less than other developing countries. So there is an urgent need to popularize vegetables and increase its production.

The definition of vegetable implies that these are edible plants which store reserve food in roots, stems, leaves and fruits which are eaten cooked or raw as salad plants. These are the great source of carbohydrate, food which is greatly present in the form of starch and occasionally as sugar, proteins etc. Some indispensable minerals, salt and vitamins also increase the food value of the

vegetable. The term vegetable is applied to the edible herbaceous plant or its parts which are commonly used for culinary purpose. Vegetables play an important role in human diet, supplying some of the things in which other food materials are lacking. The nutritional value of vegetables depends upon the edible portion of the plant that is used as food. The edible part may be bulb, bud, flower, fruit, leaf, rhizome, root or seed.

Some vegetables are eaten only raw, like radish, carrot, beet and turnip, some other like garlic and onion are eaten either raw or cooked while, some of the vegetables are eaten only after being cooked like pumpkin etc.

They may be used as appetizer (cucumber, tomato etc.) garnishing (green chillies etc.), juice (bitter gourd, tomato etc.) main dish (brinjal, pointed gourd etc.) soup (spinach; tomato etc.) sweet dish (sweet potato), salad (carrot, radish, beet, turnip etc.) and seasoning like coriander and garlic etc.

The vegetables play an important role in the balanced diet of human begins by providing not only the energy-rich food but also promise supply of vital protective nutrients like minerals and vitamins. Comparatively, vegetables are one of the cheapest source of natural nutritive foods. Some of the vegetables are good sources of carbohydrates (leguminous vegetables, tapioca, sweet potato, yams, colocasia, potato, garlic, onion, brussels sprouts, methi), proteins (leguminous

vegetables like peas and beans, leafy vegetables, garlic, Brussels sprouts), vitamin A (tomato, leafy vegetables, root-vegetables like radish, beet, carrot and turnip), vitamin B (peas and beans, garlic colocasia, tomato, asparagus), vitamin C (green chillies, turnip, Brussels sprouts, drum-stick leaves, cauliflower, cabbage, knoll kholl, bitter gourd, radish leaves and leafy vegetables), calcium and iron (all green leafy vegetables, drumstick fruits).

The extensive studies carried out by the Indian Council of Medical Research, New Delhi and the National Institute of Nutrition, Hyderabad, have revealed that the meagre intakes and low purchasing power of even low cost protective foods such as vegetables have been largely responsible for malnutrition and under nutrition in large segments of our population. Root vegetables are those garden esculents of which the nutritive part grows under the earth. This edible part may be either a root or a stem. From the ancient times roots and tubers have furnished food for man. The root vegetable crops include beet, carrot, radish and turnip. These crops develop enlarged storage organs called roots. They contain fairly large quantities of starch and the size, shape, color of skin and flesh vary greatly with the crop.

All of us are aware of the primary challenge to increase the production of food through decreased plant disease loss in order to feed growing population.

This of course has been the primary challenge throughout our history. Now however we are asked to meet this challenge in the face of significant and growing constraints broadly described as economic, social and political. Given out track record we are confident that if our success depends solely upon technology. We would melt the challenge even under the constrants of time but despite of our technological advancement in agriculture the provision of sufficient food for our selves and the developing countries like India is a course for great concern for plant pathologists of these countries with explosive population. Prevention of essential crop losses caused by crop diseases will be one of the spokes in the wheel of food production for the 6 billion people who will come to dinner in the year 2002. In this task chemicals for plant disease control, the fungicides will play an important role as the partners with other practices in the food production.

Vegetable losses between production and consumption have recently been studied seriously even in developing countries like India and attention has been paid to preserve the root vegetables during production and consumption levels. Attack by the micro-organisms is probable the most serious cause of pre and post harvest decay of root vegetables.

To prevent crop diseases efficiently a knowledge of their nature and cause is one of the fundamental importance to control them. Enormous losses are some

times sustained in fruit crops during these above phases due to fungi. The earning can easily be doubled if adequate attention is paid to the improved cultural practices and to control of the diseases of root vegetables both in field and in storage. Thus there is an urgent need for undertaking detailed studies of root vegetable diseases and advise suitable method of controlling and management of these maladies both in field and storage.

Considerable work in this fields has been done by many worker in many countries special nutrition may be made of the work of Cunningham (1928), Johnson (1949), Leclerg (1935), McKay and Pool (1918), Leonard (1940), Bockstahler (1940), Stewart (1931), Doran (1928), Hooker (1944), Thomas (1943), Wilson (1944), Groves (1944), Lauritzen (1926), Meier (1922), Wiant (1941), Atkinson (1950), Chupp (1935), Rangel (1945), Anderson (1933), Strider (1962), Standberg et al. (1972), Chupp (1953), McLean (1960), Walker (1952), Amador (1976), Wilson (1962), Henis (1978), Anderson (1952), Netzer (1966). Mukhopadhyay (1969), Narain (1978), Prabhu (1965), Shukla, Singh and Bhargava (1978) and Srivastava (1979).

Beet *(Beta vulgaris* family-Chenopodiaceae, Commen name - Chukander), the garden beet or table beet is also popular root vegetable grown in home gardens as well as market gardens mainly for its fleshy enlarged roots. In fact, beet is

useful vegetable in a number of ways. The swollen roots are eaten boiled or as a salad. They are also used as pickled. The tinder leaves and the young beet plants are used as greens (pot herbs). The large sized but are used for canning.

Beet roots are rich in Protein, Carbohydrate, Calcium, Phosphorus and Vitamin C. They are also rich in iron and vitamins (Iron 3.1 mg, Vitamin A. 21000 IU, Thiamine 10 μg and ascorbic acid 50 mg per 100 gm edible portion) (Nutrition chart, Heinz Co., 1942, p. 21). The use of beet probably dates back to the prehistoric times when the leaves were used as pot herbs. According to Anderson (1952) beet was certainly domesticated first as a leafy vegetable, then as a root crop and finally as a source of sugar. The Beet is affected by comparatively few diseases. The most serious diseases are leaf-spot, blight, root-rot, wilt and soft-rot are very common.

Carrot (*Daucus carota*, family - Umbeliferae, common name - Gajar) is a winter season crop. It is grown all over the world in spring, summer and autumn, in temperate countries and during winter in tropical and subtropical climate. The world-wide consumption of carrot has increased over the years and it is now one of the most popular vegetable crops. Undoubtedly carrot is one of the most ancient vegetables. It is pastinaca, a name latter transferred to the parsnip when carrot become carota (Burkill 1935).

Carrot is valued as food mainly because it is a rich source of α and β carotene. In carrot roots, sucrose is the most abundant endogenous sugar, 10 times higher than glucose and fructose. The last two are generally present in a 1:1 ratio although this ratio may vary considerably between caltivars.

The nutritive quality of carrot roots has been found to be influenced by nitrogen levels and sowing methods. The best quality roots are produced when sown in rows on the flat, and N at rate of 60 Kg/ha was applied (Kumar et al. 1974) Draglono (1978) showed that roots are different size (50 - 250 g) did not differ in taste or in the contents of sucrose, glucose, carotene, nitrate or mg. However the largest root contain more N, P and K and the percentage of dry matter tended to be higher.

Carrot roots are used as a vegetable for soups, stews, curries and pies; grated roots are used as salad, tender roots as pickels, Gajar halwa is a delicious dish. Carrot jam is also popular and the roots in the form of disc and slices, can be dehydrated. Carrot juice is a rich source of carotene and is some times used for colouring butter and other food articles. Carrot leaves are said to be eaten in many countries, its tops can be used as a good source for extraction of leaf protein. Moreover, carrot tops are used as fodder and also for preparation of poultry feed.

An influsion of carrot has long been used as a folk medicine for threadworms. Schuphan and Weiller (1967) noticed antibacterial property essential oils extracted from carrot roots. Carrot increases the quantity of urine and helps elimination of uric acid. Addition of large amount of carrot to the diet has a favourable effect on the nitrogen balance. Carrot seeds are aromatic stimulant and carminatine. They are reported to be useful in diseases of the kidney and in dropsy (Chopra, 1933 ; Kirtika a Bosu 1935).

The common diseases of carrots are leaf blight, root-rot, leaf-spot and wilt are very common.

Radish (*Raphanus sativus*, family - Cruciferae, common name - Muli) ; is a popular vegetable in both tropical and temperate regions. Radish is one of the most ancient vegetables. Inscriptions on the inner walls of Pyramids show that radish was an important vegetable in Egypt about 2000 B.C.. Certain remarks of Herodotus reveal that it was cultivated about 2700 B.C. (Becker 1962). Radish is a good sources of Vitamin 'C' (ascorbic acid), containing 15-40 mg per 100 g. of edible portion and supplies a variety of minerals. Trace element in radish include aluminium, barium, lithium, manganese, silicon, titanium, fluorine and iodine (up to 18 μg./100 g). Vitamin C content of radish roots is greatly influenced by light conditions. For example, found that root vitamin content was higher in plant

grown under blue light, while Lichtenthaler (1975) noted enhanced synthesis of β-carotene under red light. While Patil and Patil (1986) recorded an inverse relationship between Nicotinic acid application and Vitamin C contents in both roots and tops.

Radish contains glucose as the major sugar and smaller quantities of fructose and sucrose and pectin. The characteristic pungent flavour of radish is due to the presence of volatile isothiocyonites (trans - 4- methyl - thiobutenyl isothiocyanate). The leaves of radish are a good source of extraction of proteins on a commercial scale (Joshi 1971 ; Bagcthi et al. 1977). The radish seed are a potential source of non-drying fatty oil suitable for soap making, illuminating and edible purposes. The pod of rat-tail radish contain moisture 92.3%, protein 1.3%, fat 0.3%, fiber 1.1%, carbohydrate 4.3% and minerals 0.7%. They also contain 7.8 mg calcium and 2.4 mg phosphorus per 100 gm at edible portion. The juice of fresh leaves is used as diuretic and laxative. The seeds are said to be peptic, expectorant, diuretic and carminative (Kirtikar and Basu 1935). Some of the important diseases of Radish are root-rot and blight are very common.

Turnip (*Brassica rapa,* family - Cruciferae, common name-Shalgam) ; is an annual plant the stem is extremely reduced bearing cluster of leaves at its top, the

napiform root is edible. Turnip is an important root vegetable grown as a summ crop in temperate climate on as a winter vegetable.

The turnip root contains 41.6% moisture, 6.2 g carbohydrate, 0.5 g protei 0.2 g fat, 0.04 mg thiamine, 0.04 mg riboflavin, 43 mg ascarbic acid, 30 mg ca, 40 mg p. and 0.4 mg Fe per 100 gm of edible portion. The turnip green contains 15669 IU. vitamin A per 100 g.

The fresh roots of turnip are consumed in salad or cooked as a vegetable or used in pickles. Its young leaves, which contain high amount of ascorbic acid and iron, and rank second in vitamin 'A' content, are eaten cooked as greens. Most of the diseases that attack radish as blight, leaf-spot and root-rot are common diseases of turnip also.

Study on nutritional behaviour of a micro-organism on various substances helps in understanding its pathogenic behaviour. A knowledge of nutritional requirement of pathogenic fungi should be of aid in interpreting the phenomenon of hyphae penetration into plant tissue as well as establishment of the organism after penetration.

Three things are essential for occurrence of any disease : a favourable environment, a suitable host and a virulent pathogen. Jones (1972) initiated a new

field of research on the effect of various environmental factors upon the epidemiology of disease. He affirmed that, "The role of environment to the predis position of host as well as to the virulence of the parasite can not be over emphasized".

Temperature is one of the important external factor which influence biological systems. Fungi grow within a certain range of temperature which varies with the nature of the organism. Review on effect of temperature on fungal growth have been prepared by Hawker (1950) Cocharane (1965) and Deverall (1965). In the present study influence of temperature on growth and sporulation of above organism have been observed.

In the case of soil-borne disease which attack the seeds or seedlings. It is well known that root diseases causes considerable economic losses of root vegetables. Root disease are in several ways different from the diseases of the arial part. A knowledge of the complex microbial interactions occurring in the soil and of the ecology of soil micro-organisms, saprophytic and parasitic is essential for a proper understanding of root disease and for a rational approach to their control. In the case of soil-borne disease, in addition to the climate, the physical and chemical characteristics of the soil, especially the soil biota, play a predominant role.

Seed-borne micro-organisms have been reported to reduce storage life of seed and induce various bio-chemical changes. Christensen and Kaufmann (1965 and 1969) have categorised the fungal forms into two, on the basis of their invasion on the seed.

Seed borne fungi may be found associated externally to the seed surface or in the deep seated internally in the seed (Prabhu (1967). These fungi may prove to be pathogenic if the host and other environmental conditions are suitable. If seeds are stored at high moisture content, the fungus may survive in seed but at normal storage conditions, they have no activity or are believed to be dead due to low moisture content. Field fungi may often cause deterioration of nutritional content of seed, reduction in germination and seedling growth. Some of the common field fungi are the species of *Alternaria, Botrytis, Cercospora, Fusarium* and *Sclerotium*.

Fungi which grow on stored products are known as storage fungi. They come in association of seed with during transit and storage. These fungi grow on a variety of organic and inorganic substances, particular on decaying food products and on the decaying plant materials. They are abundantly present in atmosphere and thus serve as a source of contamination for seeds. Storage fungi usually do not invade before harvest but they may be found on the seed in low percentage,

nevertheless providing for the presence of inoculum for storage fungi (Qasem and Christensen, 1958 ; Tuite, 1959 and 1961).

One of the major factors in low production of vegetables in India is, poor seed germination and mycoflora associated with seeds and soils. Mycoflora cause damage of seeds and plant. Therefore, a detailed information about seed and soil mycoflora may be helpful in reducing their occurrence by suitable measures. Both pathogenic and non-pathogenic forms are responsible for poor seed germination, pre and post-emergence rotting of the germinating seed or reduction in the vigour of the seedlings. Various workers including Atlas et al. (1970), Gupta and Chohan (1970), Khandelwal and Prasad, (1970), Kadian and Suryanarayan (1971), Jhamaria et al. (1975) and Arora (1987) have studied the seed mycoflora of different seed. Therefore, in the present study, an attempt has been made to distinguish the pathogenic forms and test their pathogenic capability.

Among the micro-organism, fungi play a significant role in deteriorating the quality of the seed during storage which were kept for utilization of sowing purpose (Tervet, 1945 ; Tuite and Christensen, 1955 ; Armolic, et al. 1956 ; Christensen, 1957 ; Diener, 1960 ; Richard et al. 1962 ; Nicholsan and Sinclair, 1973 ; Whittle, 1977 ; Vidyashekaran et al., 1980 ; Bhadraih and Ramarao, 1987

and Rashmi and Mehrotra ; 1990). An attempt was therefore, made to investigate the mycoflora in storage.

Moisture content and temperature are the main factors determining the development of fungi. Slight difference in moisture content make a great difference in the growth rate of fungi. Many workers including Kennedy (1964), Christensen and Dorworth (1966), Christensen (1967 and 1972) and Jayaraman and Kalyansundaram (1989) have tried to correlate the moisture content of seed and the invasion of fungi in different seeds by very little work has been done with vegetable seeds (Singh, 1986 ; Mohanty, 1981 and Shukla, 1987). Hence, present investigation was aimed to study the fungal association of various vegetable seeds in relation to moisture.

As primary infection comes from the infected seed, experiments were carried out to control seed-borne fungi by trying various chemotherapeutants. Earliar, various workers have tried to control the fungi, in Vitro (Lilly and Barnett, 1951 ; Srivastava and Tandon 1971; Miller, 1972 ; Rajagopalan and Wilson, 1972; Haware and Joshi, 1974; Kumar and Kumar, 1980 ; Tiwari, 1986 and Dwivedi, 1990). Hence, it was essential to evaluate various chemotherapeutants against different seed-borne fungi.

Planning for disease control involves strategy and tactics and demands a knowledge of the chemical use for controlling plant disease. Interest in use of fungicides in plant disease control have vastly increased. Their use in controlling various diseases of fruits and fruit crops have been suggested by numerous workers in last few years, viz., Spalding and Reeder (1972), Bhargava and Singh (1975). In order to economies and avoid wastage of fungicides it is pre-requisite to evaluate the fungicides in laboratory before testing them in field conditions. It not only saves time but also prevents the wastage of ineffective ones. In the present study, therefore, an attempt has been made to evolve suitable methods for controlling the diseases under investigation.

For the production of disease free and healthy crops, seeds of different vegetables were treated with various chemotherapeutants by several workers (Radhalkar and Neergaard, 1969; Nene and Srivastava, 1971; Srivastava and Tandon, 1971; Grover and Dutt, 1972; Chakravorti and Anil Kumar, 1975; Byford, 1977). The seeds of different vegetables included in the present study have been treated with effective chemical and suitable control measures have been suggested.

Biological control is the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active dormant stage, by one or more

organisms, accomplished naturally or through manipulation of the environment, host or antagonist, or by mass introduction of one or more antagonists. Biological control rarely eliminates a pathogen from the site but rather reduces its number or its ability to produce disease. Such control may be achieved with little or no reduction inpopulation of the pathogen, or perhaps without preventing infection. In the present investigation biological control of plant pathogen of root vegetables, was undertaking to find-out an effective biological control method including effective bio-chemicals.

The present problem was undertaken as an integral part of a comprehensive plan of experimental investigation, complying organized studies for the furtherance of scientific knowledge in this important and complex fields of research. The investigation were conducted with recognized methodology.

A comprehensive survey of various cultivated area of root vegetable of Allahabad, Varanasi, Sant Ravi Das Nager Jaunpur, Pratapgarh of Uttar Pradesh, was made in which these root vegetables i.e. Beet (*Beta vulgaris*), Carrot (*Daucus carota*), Radish (*Raphanus sativus*) and Turnip (*Brassica rapa*) were studied, observation from various surveys revealed that about 10 to 60 percent root vegetables were affected from fungal disease and great losses occurred due to disease.

Disease symptoms produced on the respective hosts were carefully studied. The causal organism was isolated from its host. Its pathogenicity and identity established when isolations were made from the seed and soil of the infected fields.

Fungi isolated, cultured, purified maintained and identified. Effect of moisture content present in different seeds of root vegetables and percentage of seeds contaminated with fungal flora has also been observed. Pathogenicity tests have been carried out with these organisms. Symptoms produced recorded on their respective host work.

Fungicides have been evaluated in the laboratory against some pathogenic forms and the successful one have been further tried in the epiphytatic field conditions.

An attempt has also been made to investigate the inter-relationship between certain root vegetable seeds and their seed borne and field fungi.

CHAPTER - 2

MATERIAL AND METHODS

MATERIALS AND METHODS

Seed and disease (root, stem and leaves) samples of root vegetables viz. Beet (*Beta vulgaris*), Carrot (*Daucus carota*), Radish (*Raphanus sativus*) and Turnip (*Brassica rapa*) were collected from Allahabad, Jaunpur, Varanasi, Sant Ravi Das Nager and Pratapgarh district in U.P.. The specimens were brought to the laboratory in separate clean polythene bags. Collected materials were surface sterilized with 90% ethanol and few cut from the junction of the healthy and infected parts were transferred aseptically to Petri-dishes containing PDA.

The fresh stock of culture was maintained by subculturing the fungus at regular intervals of three week on Potato-Dextrose-Agar medium and the also where it was necessary single spore culture of the fungi were prepared with the help of a dummy cutter objective. The following media were used for isolation and other studies.

Malt Extract Agar medium

Malt extract	20 g
Agar - Agar	20 g
Distilled water	1000 ml

Czapek's Dox Liquid medium :

$NaNO_3$	2.00 g ;
K_2HPO_4	1.00 g.
KCl	0.50 g.
$MgSO_4$, 7 H_2O	0.50 g.
$FeSO_4$,. 7 H_2O	0.01 g.
Sucrose	30.0 g.
Distilled Water upto	1000 ml

Asthana and Hawker's medium 'A'

Glucose	5.0 g.
KNO_3	3.5 g.
KH_2PO_4	1.75 g.
$MgSO_4.7H_2O$	0.75 g.
Distilled water	1000 ml.

20 g. Agar - Agar was added to solidify.

Potato Dextrose Agar medium

Potatoes (Peeled and sliced)	200 g
Dextrose	20 g
Agar - Agar	20 g
Distilled water upto	1000 ml
pH	6.0 - 6.5

The potatoes were cooked for 30 minutes in a flask containing 500 ml of distilled water. The potato juice was decanted into the melted Agar and the volume was adjusted to 1000 ml with distilled water. Dextrose was then added and the medium was sterilized at 15 lb pressure for twenty minutes.

To study the occurrence of the pathogen/pathogens in non-rhizosphere, rhizosphere and rhizoplane regions of the diseased plants, soil samples were collected in sterilized polythene bags with sterilized spatula. They were brought to the laboratory and soils of replicate samplings were mixed thoroughly. Mycoflora were isolated on nutrient media by dilution plate techniques.

The method used for collecting soil samples was similar to that used by Saksena and Mehrotra (1952). Soil from various depths (5, 7.5, 10, 15 cm) were scraped from the four sides of the pit using a sterilized sharp steel blade, the sample collected from various depths of a locality were then mixed together thoroughly. The sample of each locality were packed separately in pre sterilized polythene bags and brought to the laboratory were the sample were air dried pulvarised and passed through a sieve (2mm)

The soil dilution and plate count method were used to determine the number of fungus colonies in a particular sample. Modified Czapek-Dox Agar medium (Singh and Nene, 1965) containing sodium nitrate ($NaNO_3$) 2.0 g,

dipotassium monohydrogen phosphate (K_2HPO_4) 1,0 g, Magnisium sulphate ($MgHO_4$ 5 g., $FeSO_4$) 0.1 g., Sucrose 30 g, Agar 20 g and distilled water to make up 100 ml was used. After autoclaving fresh water solution of malachite green (50 mg/l) and captan (100 mg/l) was added. The medium was left to cool a little and then 20 ml of the medium was poured into each sterilized plate and left to solidify. The dilution (1:1000) was added to the petridishes containing the solid medium. These petridishes were then incubated at 25°C ± 1°C for 3-5 days.

Samples of seeds were stored in sterilized widemouth stoppered glass bottles at 4 ± 2°C. The collected samples were 'composited' and reduced to a 'Submitted sample'. A 'Sub sample' from the submitted sample was taken as 'Working-sample' for every individual variety.

For the analysis of seed-borne mycoflora, following two methods were employed for screening maximum number of fungal propagules :--

— Standard blotter method (ISTA, 1966)

— Agar plate method (Muskett and Malone, 1941)

Degree of infection of a fungus in a sample was determined on the basis of grains showing its association and was expressed in terms of percentage infestation (Srivastava, 1992)

$$\%\text{Infection} = \frac{\text{(Number of seeds showing association of fungus in a sample)}}{\text{Total Number of seeds used}} \times 100$$

Three layered circular moistened blotting paper with distilled water were put in the Petri-dishes and were subsequently sterilized along with Petri-dishes in an autoclave at 15 lb presure for twenty minutes. One hundred seeds of every sample were subjected for their analysis of fungal flora associated with the seed samples.

In order to isolate the internal mycoflora, it is necessary to dissociate the external mycoflora by dipping the seeds for two minutes in 0.1% mercuric chloride solution. Thereafter, the seeds were thoroughly washed with sterilized distilled water to remove any trace of mercuric chloride before plating them. Rest of the process is same as described earlier.

Twenty ml of sterilized Potato Dextrose Agar medium (Riker and Riker, 1936) supplemented with aqueous streptomycin as antibacterial agent was poured in sterilized petridishes. One hundred seeds of every sample at the rate of five seeds per plate were screened for their respective mycoflora. The experiment was set at 25 ± 1°C for seven days. After this, the percentage incidence of fungal

colonies associated with the seeds were recorded and transferred to culture tubes containing Potato Dextrose Agar slants.

Appearently healthy seeds were surface sterilized and rolled thoroughly over well sporulated cultures of different fungi which are sown in sterilized earthen pots, previously filled with sterilized field soil at the rate of twenty five seeds per pot. One hundred seeds were taken in each case. Equal number of unsterilized and surface sterilized (control series) seeds were sown in earlier mentioned way. After 15 days of experiment, percentage pre and post-emergence mortality and the vigour of the seedlings which survived were recorded.

Various test fungi were grown in 100 ml of sterilized medium already kept in Erlenmeyer flasks and incubated at 25 ± 1°C for 15 days. The culture medium was filtered through Whatman No. 42 filter paper and the filterate was collected seperately and labelled as 100 percent pure.

The composite seed samples were stored in sterilized glass container for a long period of three years under laboratory conditions. The mycoflora associated with these seeds were isolated and studied at regular intervals of six month, from unsterilized and surface sterilized seeds using Agar plate and standard blotter methods (as formerly described). The percentage incidence of different fungal

forms and percentage germination of stored seeds at different intervals of time were recorded.

In order to study the effect of moisture content on fungal flora, 10g. seeds of each samples were taken. To study the percentage moisture content, seeds were dried in oven at 105°C for 48 hrs. Seed containers were subsequently kept in the dessicator for cooling and weighed again. Moisture content of the seeds was calculated by the formula given below :

$$\text{Percentage moisture content} = \frac{W - W_1}{W} \times 100$$

where,

W = fresh weight of seed sample

W_1 = dry weight of seed sample

After the determination of initial moisture content, the seeds were adjusted to the desired moisture content by adding the required amount of sterilized distilled water and left over-night. The moisture content was again recorded after drying the seeds in oven (Jayraman and Kalyanasundaram, 1989).

Seeds were plated on blotters (without surface sterilization) for analysis of its mycoflora. The total number of colonies present in a particular seed variety were recorded.

The cultures were made bacteria free by the method described by Brown, 1924. Stock cultures were maintained on Potato Dextrose Agar medium. Pyrex glasswares and extra pure reagents supplied by B.D.H., E. Merck, Difco were used throughout the present investigation.

The culture media were sterilized by autoclaving at 15 p.s.i. for 20 minutes. Petri-dishes were sterilized on electric oven at 160°C for 4 hrs. Experiments on the effect of pH and different temperatures indicated that pH 6.5 and a temperature of 25°C ± 1°C were optimum, both for the growth and sporulation, for the present fungi. It was, therefore, decided that for physiological studies the pH of all the culture media is adjusted to pH 5.5 and temperature maintained at 25°C ± 1°C. Sodium hydroxide and hydrochloric acid were used for adjusting the pH. In order to determine the dry weight of these organisms, 25 ml of liquid medium was taken in 150 ml Erenmeyer conical flasks. The solutions were autoclaved at 15 p.s.i. for 20 min but in some cases fractional sterilization had to be carried out and this is indicated at appropriate places in the text. Garrette (1936) Agar disc method was used for inoculation. 7 to 10 days old cultures were used for this purpose. In a few

experiments spore suspensions containing similar amount of spores was used. Unless otherwise mentioned three replicates were taken for each treatment.

The fungal mats were harvested at the end of the incubation period by filtering over previously dried and weighed Whatman's filter paper No. 42. They were then washed with distilled water and were dried to constant weight at 65°C after which they are transferred to a dessicator, cooled and rapidly weighed on an analytical balance. An open bottle of calcium chloride was kept inside the balance to absorb atmospheric moisture while weighing. In general, it was found that there was no significance difference between the replicates and hence, only the average values of the three replicates have been recorded and they have been used as quantitative measure for comparing the growth of the fungi under various treatments. The change in the pH after incubation period was also recorded by B.D.H. pH papers.

Mode of spore germination and effect of various factors viz., effect of environment, fungicides and leaf extracts of various Indian medicinal plants on spore germination was studied using Hofman's (1860) hanging drop technique.

To obtain the leaf extract, expressed juice of thoroughly washed fresh leaves of medicinal plants were filtered through two layers of clean cheese cloth and finally through Whatman filter paper No. 42 to get a clear filtrate. To prepare

50%, 75% and 100% concentrations of extracts required amount of distilled water was added to the stock solutions.

The evaluation of various fungicides, on the basis of their inhibitory effect on the vegetative hyphe by the method recommended by Forsberg (1949) was followed. Small pieces of sterilized cotton threads (about 2 cm in length) were placed on Asthana and Hawker's medium 'A' in Petri-dishes, which were separately inoculated with different organisms under study. The thread which got covered with the vegetative hyphae of the fungi within a week were subsequently rolled in dry fungicides or momentarily dipped in the liquid fungicides. The treated threads were transferred to another set of Petri-dishes containing Asthana and Hawker's medium 'A' and were incubated at 25°C ± 1°C for 7 to 10 days. Those fungicides which did not allow the pathogen to grow were considered effective against a particular pathogen. Their effective concentrations were also investigated and they were later tried on stem and roots. For this purpose, poisoned food technique (Nene and Thapaliyal, 1979) was applied. In this, the required amount of each chemicals was mixed with known amount of sterilized PDA medium before solidification and then it was poured into sterilized Petri-plates, Pathogens including *Alternaria radicina, A. dauci, A. raphani, Botrytis cinerea, Cercospora beticola, C. carotae, Fusarium oxysporum* and *Sclerotium rolfsii,*

were inoculated in the centre of each Petri-dish. In case of control series, no pathogen was inoculated. The growth of different pathogens were recorded after 7 days of incubation at 25 ± 1°C. Absence of fungal growth at any concentration gave the minimum concentration required to control the pathogen in-vitro.

On the basis of in-vitro study, effective fungicides were tried on seeds and soils. There concentrations viz., 500, 750 and 1000 ppm solution were prepared in each distilled water in sterilized flasks and seeds were treated with each concentration. The seeds were soaked for few hours in different concentrations of fungicides prepared according to their effective ingredients. The seeds were then plated in Petri-dishes containing three layered moist blotters and incubated for seven days at 25 ± 1°C. Control sets were also maintained where seeds were soaked in sterilized distilled water replacing the antibiotics and fungicides. The percentage seed germination and associated mycoflora were recorded.

Plant leaf-extracts were prepared by the following :

Cold distilled water extraction : A 50g of leaf was washed, chopped and crushed using mixie with 100 ml. of distilled water and filtered through muslin cloth. Then the filtrates were used for inhibition zone technique by paper disc method and poisoned food technique.

Hot distilled water extraction : In this method distilled water at 70°C was used instead of cold distilled water. Then these extracts were used for inhibition zone technique by paper disc method and poisoned food technique. The leaf material used for cold distilled water extraction alone was used for hot distilled water extraction at the same concentration.

Testing fungitoxicity of the plant leaf-extracts, the method was slightly modified and carried out. Sterilized filter paper discs (10 mm) were soaked in cold and hot distilled water plant extracts and 50% concentration. In each of the Petri-plates two treated discs were placed at the centre on potato dextrose agar medium. Suitable controls were maintained by placing the discs soaked in sterile water. The mycelial discs (9 mm) were kept at three places on the periphery of Petri-plates at equal distance. Each treatment was replicated three times and incubated at room temperature (28 ± 2°C) for 24 hours.

The effect of plant leaf-extracts on the growth of the pathogen was studied by poisoned food technique. A 50g of different plant leaf materials extracted in 100 ml. of hot and cold distilled water were used. Five ml. of these extracts were taken to incorporate into 50 ml. of potato dextrose agar medium and autoclaved for 20 minutes at 15 psi/inch^2 pressure. The plant leaf-extract incorporated medium at 5% was poured into the sterilized petriplates and allowed to cool. The plate were

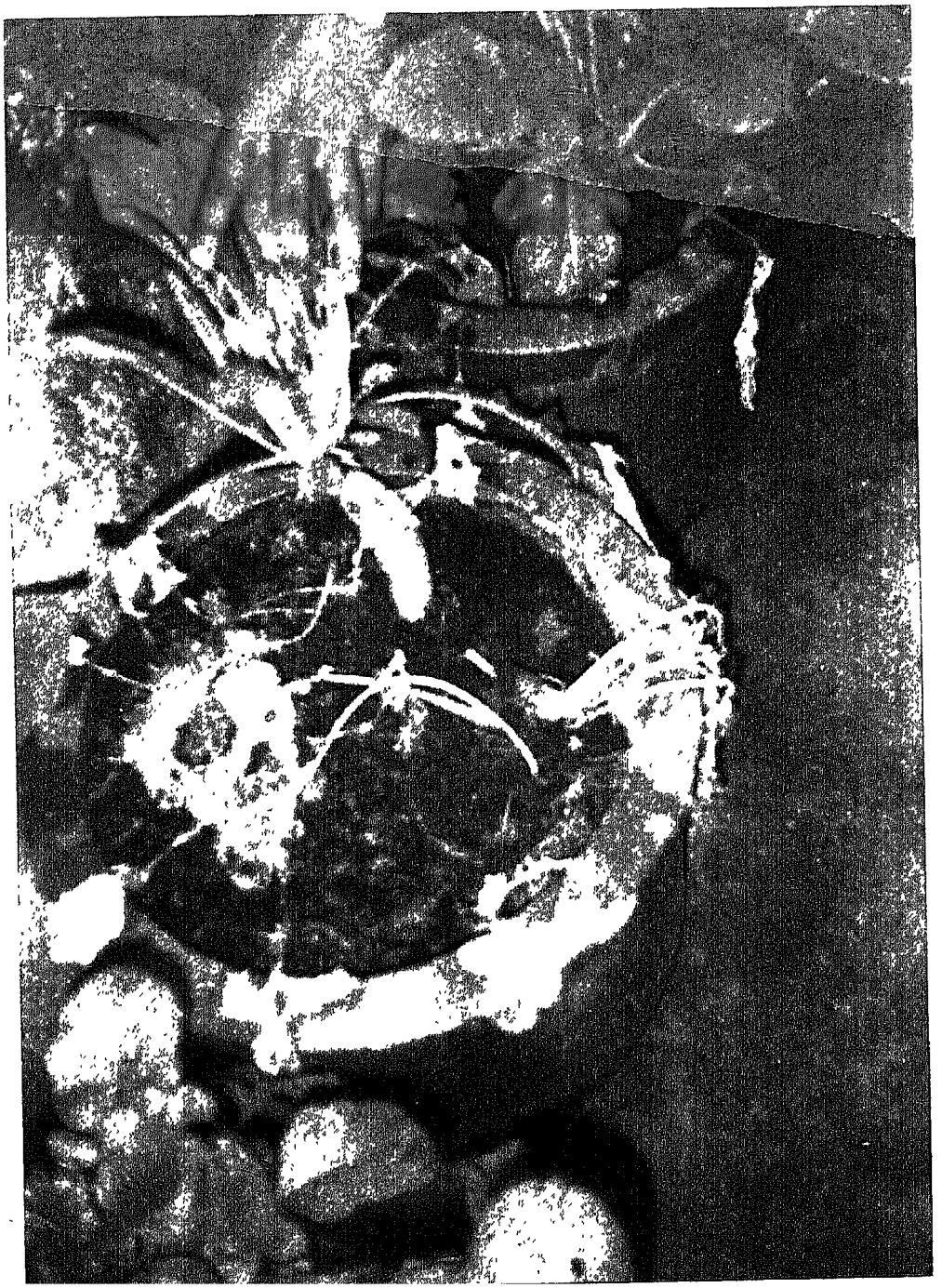
inoculated with uniform discs of 9 mm diameter from 3-day old culture grown on Potato Dextrose Agar medium the diameter of colony growth was recorded after 24 hours and the mean inhibition percentage worked out. The inoculated Petri-plates were incubated at room temperature (28 ± 2°C) for 24 hours with suitable control. The treated and sterilized water soaked seeds were kept for germination in roll towel and each treatment was replicated three times. The seeds were kept for germination at 25 ± 1°C and 90 ± 2 percent relative humidity.

Plant leaf-extracts tested under roll towel method were applied on the root vegetable seeds at 50% concentration for 6 hours and then shade dried for 2 hours. Pathogen multiplied on sand maize medium (19 : 1 ratio) was used for the inoculation of unsterilized soil. The inoculum was mixed at the rate of 5% to the soil and it was incorporated one day before sowing. Each treatment was replicated three times. One hundred seeds were used for each treatment and the observations were recorded. Similar experiment was also conducted to assess the efficacy of storage seed treatment on the seedling mortality.

Pathogens multiplied on sand maize medium were incorporated into the unsterilized soil one day before sowing at the rate of 5g per 100g of soil in earthen pots. Root vegetable seeds were sown in inoculated soil and the plant extracts used for seed treatment were also used for soil drenching at 25% concentration immediately after sowing. Drenching the soil was given upto 5 cm depth. Seeds sown in pathogen inoculated and uninoculated control were also drenched with tap water. Each treatment was replicated three times. One hundred seeds were used for each treatment.

CHAPTER - 3

ISOLATION STUDIES



ISOLATION STUDIES

The micro-organisms associated with seeds and soils are serious parasites of seed primordia and maturing seeds affecting them both qualitatively as well as quantitatively resulting poor seed-germination and diseases of seedlings in the field. More attention is beging paid to study of the seeds and soils mycoflora in view of their importance as deteriorating agents. A large number of fungi were found to be commonly associated with the seeds of vegetables causing considerable damage either directly to the seeds or crops that are raised from these contaminated seed stocks. It was, therefore, considered desirable to study in detail the fungal flora of the seeds of four different root vegetables namely, *Beta vulgaris, Daucus carota, Raphanus sativus* and *Brassica rapa.*

Fungi were isolated from the seeds (unsterilized and surface sterilized) soils and diseased plant parts, by means of Agar plate and blotter methods. The results are recorded in Table 1 and 2.

In common diseases of seed borne of root vegetables, the pathogen may be mixed with seed lot. Since seeds are good substrate for fungi, they build up their inoculum potential in the seeds during germination and even kill the seedlings. Establishment of seed infection is more complicated. Many factor particularly

physiological conditions, the pathogen and the host in conjunction with weather conditions are responsible for it. A few seed borne pathogens of root vegetables have been thoroughly investigated with regard to the precise cause of transmission, including establishment of infection and development of disease in the subsequent vegetable.

It is evident from the Table-1 that twelve genera viz.; *Alternaria, Aspergillus, Botrytis, Cercospora, Curvularia, Fusarium, Helminthosporium, Rhizoctonia, Sclerotium, Mucor, Phoma* and *Rhizopus* were islated from the seeds, soils and disease plant parts of root vegetable crops.

Isolation studies showed that in the case of root vegetables, maximum association of *Alternaria, Botrytis, Cercospora, Fusarium* and *Sclerotium sp.* observed in the treated as well as untreated seeds and soils. Other fungi isolated from the untreated seed were species of *Aspergillus, Helminthosporium, Curvularia, Rhizoctonia* and *Mucor.*

Table No : 1
Fungi isolated from seeds and infected plant parts of root vegetal

Vegetables	Untreated seed	Treated seed	Diseased parts
Beet (*Beta vulgaries*)	*Alternaria alternata*	*Alternaria sp.*	Leaf
	A. ricini		
	A. tencissima		
	Aspergillus flavus		
	A. fumigatus		
	A. niger		
	A. terreus		
	Botrytis cinerea	*Botrytis cinerea*	Root
	Cercospora beticola		
	Curvularia lunata		
	C. robusta		
	Fusarium equiseti	*Fusarium equiseti*	Root
	F. oxysporum	*F. oxysporum*	Root
	F. semitectum	*F. semitectum*	Root
	F. solani	*F. solani*	Root
	Helminthosporium spp.		
	Rhizoctonia spp.		
	Slcerotium rolfsii	*Slcerotium rolfsii*	Root
Carrot (*Daucus carota*)	*Alternaria alternata*	*Alternaria alternata*	Leaf
	A. dianthicola		
	A. raphani		
	A. radicina	*A. radicina*	Root & Le
	A. dauci	*A. dauci*	Leaf
	Aspergillus clavatus		
	A. fumigatus		
	A. niger		
	Botrytis cinerea	*Botrytis cinerea*	Root
	Cercospora carotae	*Cercospora carotae*	Leaf
	Curvularia andropogonis		
	C. lunata		
	C. robusta		
	Fusarium oxysporum	*Fusarium oxysporum*	Root
	F. roseum	*F. roseum*	Root
	F. solani	*F. solani*	Root
	Helminthosporium sp.	*Fusarium sp.*	Root
	Rhizopus stalonifer		
	Sclerotium rolfsii	*Sclerotium rolfsii*	Root

Radish (*Raphnus sativus*)	*Alternaria alternata*	*Alternaria alternata*	Leaf
	A. dianthiocola		
	A. raphani	*A. raphani*	Leaf
	A. brassicae	*A. brassicae*	Leaf
	Aspergillus clavatus		
	A. flavus		
	A. niger		
	Botrytis cinerea		
	Curvularia lunata		
	C. robusta		
	Fusarium oxysporum	*Fusarium oxysporum*	Root
	F. roseum	*F. roseum*	Root
	Helminthosporium sp.	*Fusarium solani*	Root
	Mucor mucedo	*Fusarium sp.*	Root
	Rhizoctonia solani		
	Sclerotium rolfsii		
Turnip (*Brassica rapa*)	*Alternaria alternata*	*Alternaria alternata*	Leaf
	A. dianthiocola		
	A. brassicicola		
	A. brassicae		
	A. raphani	*A. raphani*	Leaf
	Aspergillus clavatus		
	A. flavus		
	A. niger		
	Botrytis cinerea	*Botrytis cinerea*	Root
	Curvularia andropogonis		
	C. robusta		
	Fusarium oxysporum	*Fusarium oxysporum*	Root
	F. solani	*F. solani*	Root
	Helminthosporium sp.		
	Mucor mucedo		
	Sclerotium rolfsii	*Sclerotium rolfsii*	Root
	S. minor		
	Phoma lingam		
	P. glomerata		

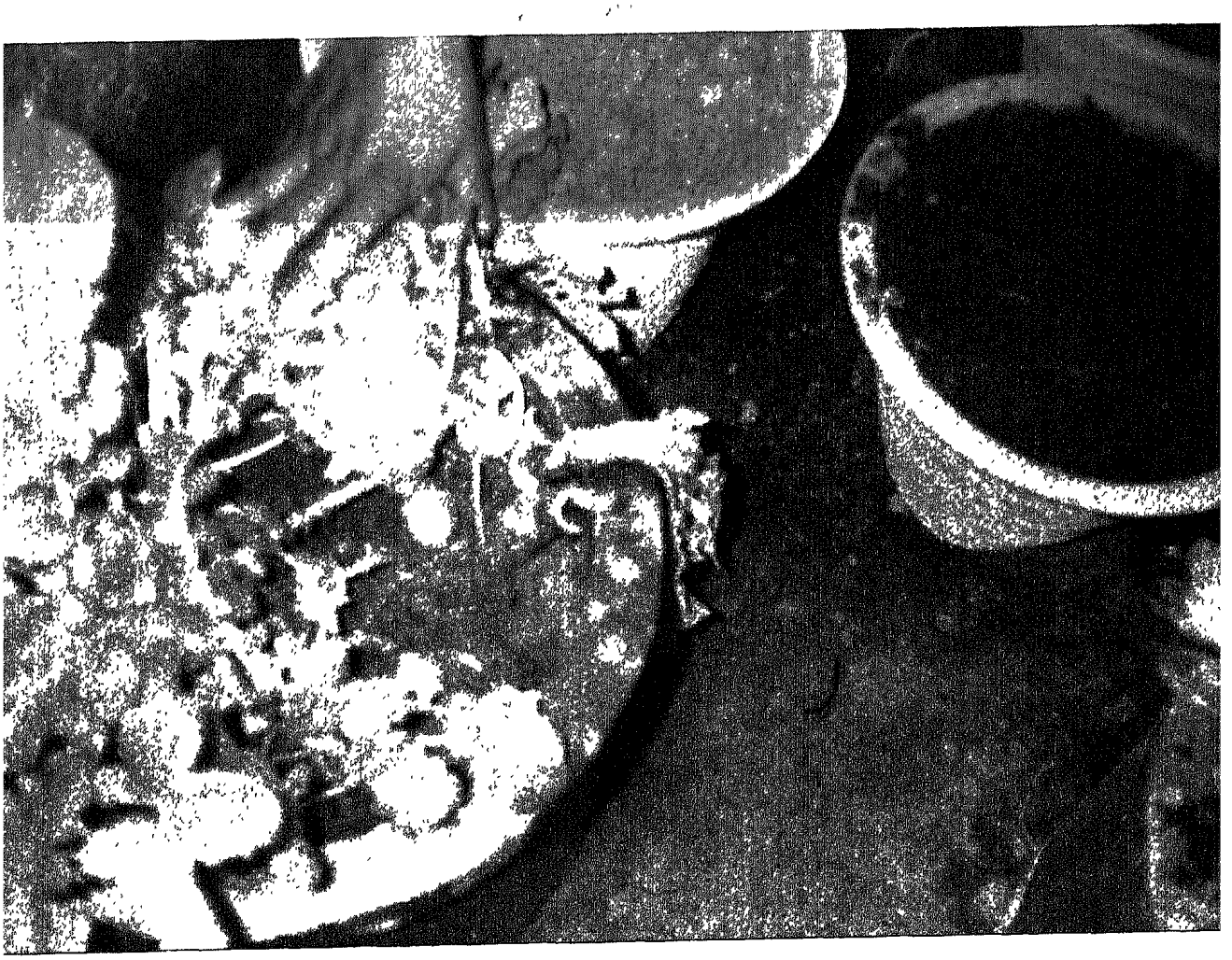
Table : 2
Fungi isolated from soils of root vegetable fields

Name of vegetable fields	Fungi isolated
Bhidiura, Allahabad	*Alternaria alternata*
	A. raphani
	Botrytis cinerea
	Fusarium oxysporum
	F. solani
	F. roseum
	F. species
	F. species
Zapharabad (Jaunpur)	*Alternaria species*
	Botrytis cinerea
	Fusarium oxysporum
	F. species
	F. solani
	F. species
	F. equiseti
	Sclerotium species
	Sclerotium rolfsii
	Rhizoctonia species
Jahnaipur (Pratapgarh)	*Fusarium oxysporum*
	F. species
	F. solani
	F. roseum
	F. species
	Botrytis cinerea
	Sclerotium rolfsii
	Sclerotium species
Mirzamurad (Varanasi)	*Botrytis cinerea*
	Fusarium oxysporum
	F. species
	F. solani
	F. species
	F. semitectum
	F. equiseti

Kanwal (Sant Ravi Das Nager)	*Botrytis cinerea*
	Fusarium species
	F. species
	F. solani
	F. species
	F. oxysporum
	Sclerotium rolfsii
	Sclerotium species
	Rhizoctonia species

CHAPTER - 4

PATHOLOGICAL STUDIES



PATHOLOGICAL STUDIES

The pathogen is an agency which generates suffering in plant and the pathogenesis is the process for the appearance of particular disease and includes the action of pathogen. The susceptibility of the host and the impact of the ancillary factors. That quality or the ability of pathogen to generate diseases is the pathogenicity which is an abstract term that carries no implication of how disease is caused. To prove that certain organism causes a specific disease, it is necessary to establish the Koch's pastulates i.e. isolation of suspected pathogen from the infected plant tissue in order to establish the indentity in culture. The culture is inoculated on other healthy plant develop the same type of disease symptom and re-isolation of organism from the artificially inoculated plants with indentical characters.

Seeds of root vegetable often become infected through pods and fruits and infections involve the seed coat and usually also a considerable part of the cotyledons (the site of infection in the fleshy cotyledons which form a rich nutritious base for the seedling as well as for pathogens) may essentially play a key role in transmination of these pathogen. According to Neeragard (1977), a

disease is borne in the seed, in the sense that potentially it is brought forth or given support by the seed.

Pathogenicity tests were performed by rolling surface sterilized seeds on sporulating cultures of the isolates and planting them on sterilized moist blotter paper in Petri-dishes as well as planting them in pots filled with sterilized field soil. Suitable controls were also maintained. Cleaned test tubes with 10 ml. tap water were taken. A sheet of Whatman No. 42 filter paper was placed over the end of a wooden plug (which can easily go inside the test tubes). The sides of the filter paper was pressed down and the rolled around the wooden plug. The rolled paper was then pushed down into the test tube, leaving the platform out of the water. Tubes were plugged with cotton and sterilized. Various isolates were grown on P.D.A. Fungal discs of 5 mm. were cut with the help of a cork borer. Such discs were placed at the centre of the filter paper platform. Earlier surface sterilized seeds of desired host were germinated and when their length was about 0.5 cm in length they were asceptically transferred to the filter paper platform. Tubes were labelled and placed on the tube stands for 10 days. Six replicates were taken in each case.

Aqueous mycelial and spore suspensions of the organisms prepared from 7 to 10 days old cultures was sprayed on injured as well as un-injured flowers, leaves and roots of their respective host plant with the help of hand atomizer.

Pods on plants were inoculated by pin-pricked injury method and sparying the spore suspension. Humidity was maintained by covering it with a polythene bag with some sterilized water at the base. Plucked pods were similarly inoculated and kept under moist glass chambers. Suitable controls were maintained for each treatment. In each case similar conditions such as amount of inoculum water and same kind of soil etc. were provided.

Fungi which were found to cause various rots (seed-rot, root-rot, flower-rot, pod-rot), wilt, leaf-spot and leaf-blight, the symptoms produced by them are recorded in the Table-3.

The same species of *Alternaria, Fusarium, Cercospora, Sclerotium, Botrytis* which commonly isolated from seeds of root vegetables, were taken for pathological studies. These studies were carried out from the same stock cultures and hence the method used for isolating, sub-culturing etc., were similar to those described earlier. For pathogenicity test of root vegetables seedling of same size and age (5 Weeks old) were taken pathogenicity test were carried out by following method :

1. The seedling root both uninjured and injured (10 injuries by sterilized needle per root) were dipped in spore suspension (about 100 spores per lower field of compound micro-scope) of different root vegetable pathogen species. The seedling were then re-planted in plastic pots. Controls were simultaneously maintained.

2. The seedling were kept in culture tubes containing 20 days old culture filtrates of different fungal species of root vegetables. In case of control, seedlings were kept in sterilized distilled water, daily observation were made.

3. Re-isolations were always made in order to confirm the infection with particular fungal species of root vegetable diseases. Ten seedlings per treatment were taken in each case. The results of both set of experiments are summarized in the Table-3.

Pathological studies revealed that *Alternaria sp.* cover Leaf-blight and *Cercospora* caused by leaf-spot. *Botrytis, Sclerotium* caused root-rot and *Fusarium* caused wilt and root-rot of root vegetable. In case of blight, spot, rot wilt, infection appears to be seed borne as the disease could also occur in infested soil.

Result from the above pathogenicity test clearly show that all the species of fungal flora were capable of causing disease of root vegetable and seedlings. Pathological studies revealed that *Fusarium oxysporum* caused root-rot, wilt, seed-rot and seedling blight of *Beta vulgaris, Daucus carota, Raphanus sativus* and *Brassica rapa. Botrytis cinerea* and *Sclerotium rolfsii* caused root-rot of rot vegetables. Leaf-spot of *Beta vulgaris* and *Daucus carota* caused by *Cercospora beticola* and *Cercospora carotae. Alternaria* caused blight and rot of *Daucus carota.* Blight of *Raphanus sativus* and *Brassica rapa* caused by *Alternaria raphani.*

Table : 3

Pathogenicity Test

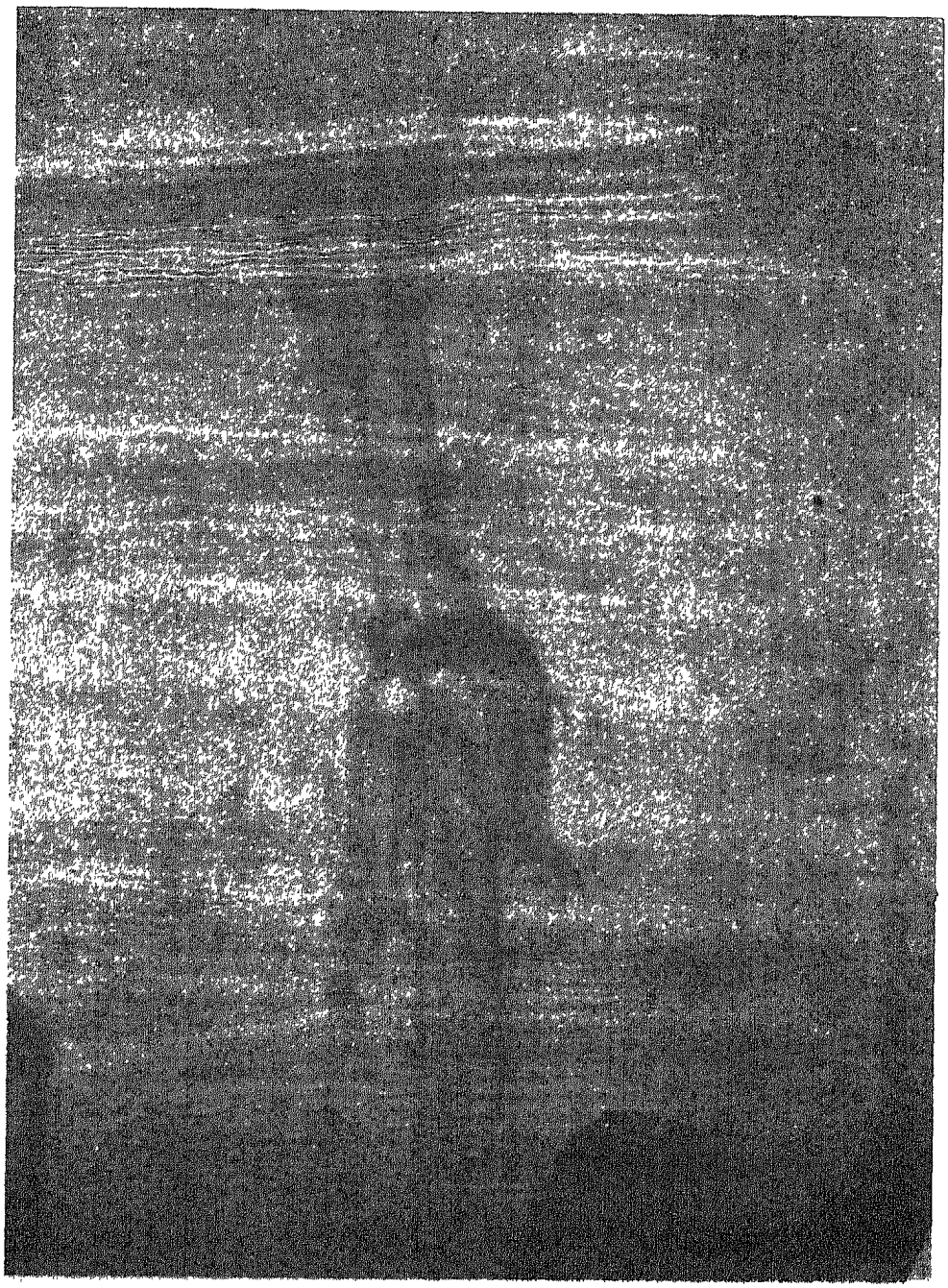
Infected Root Vegetable	Pathogen	Disease	Symptoms
Beta vulgaris	*Cercospora beticola*	Leaf-spot	Small circular spot enlarge to about 2mm in diameter often in great numbers. They have definite margin often deeper in colour than the surrounding tissue. The center tissue turns brown and as sporulation occurs, it assumes a grayish cast. Petioles are also affected, the spots tending to be elongate. When spot are numerous, the whole leaf becomes senescent dies pre-maturely and drops. Older leaves are the most readily affected and when condition are favorable for epidermic development, progressive blight and defoliation occur while young leaves continue to grow. The result is corresponding reduction in root growth and yield and in sugar content of sugar beet. On seed plant all above-ground parts affected, including the seed cluster.
Daucus carota	*Cercospora carotae*	Leaf-spot	The first signs usually appears as elongate lesions along the edge of the leaf segment resulting in a lateral curling as growth of the host continues. Non-marginal lesions appear as small, pin-point chloratic spots which soon develop into a necrotic center surrounded by a diffuse chloratic border. In periods of relatively low humidity the spot tend to be light tan in color.

			Coalesence of spot is common. linear dark lesions develop on the petiole, sometime gridling the later and killing the leaf. In humid weather the spot is darken in colour, but the necrotic tissue takes on a gray cast as the fructification of the pathogen takes place.
	Alternaria dauci	Blight	The symptom much like and often confused with those of the latter disease. The lesion are usually more irregularly shaped, and the necrotic tissue is more consistently dark brown to back. The chloratic area surrounding the necrotic tissue is usually more pronounced. Fungal is inclined to attack older rather than younger leaves and thus commonly the later of two appear.
	Alternaria radicina	Black rot of carrot	The symptoms on the leaves and petiols are not distinguishable from those of alternaria blight. In general the lesion of the later are the more widely distributed over in foliage. On roots the lesions appear usually after the crop has been placed in storage they are irregulars to circular in out-line, slightly disperssed, usually not more than 3 mm depth. The decayed tissue is greenish black to jet black. Sporulation on the fungus may be evident consisting chiefly of concdiophores and conedia.
Beta vulgaris *Daucus carota* *Raphanus sativus* *Brassica rapa*	*Botrytis cinarea*	Rot	Affected tissue is water soaked and light brown and later becomes spongy. The fungus appears in later stage of decay as a white mould upon which grey mount.
Raphanus sativus *Brassica rapa*	*Alternaria raphani*	Blight	Leaf spot appears first on the seed plant on cauline leaves as yellow raised spots, which become spherical to ellitical, thin,

			translucent, with raised borders, upto 1 cm in diameter. Black sporulation may occur on the lesion and the center of the latter may drop out. Lesions appear on the root, crown, stem and pods, the last becoming shriveled and dark.
Beta vulgaris *Daucus carota* *Raphanus sativus* *Brassica rapa*	*Fusarium oxysporum*	Seed-rot, Wilt & Root-rot	The disease usually becomes noticeable as a relatively sudden wilt. Yellowing beginning on the old leaves and progressively on younger ones is the first sign of disease. A marginal inrolling and a unilateral apical twisting of young leaves follows. Older leaves die pre-maturity and drop in the fleshy root grey to brown discoloration and rot of the vascular element occurs, usually unilaterally, the discoloration often being traceable to one or more lateral roots.
Beta vulgaris *Daucus carota* *Raphanus sativus* *Brassica rapa*	*Sclerotium rolfsii*	Rot	The damage is due to a rapid semi-watery decay of the fleshy root. By the time above ground symptoms are evident, the fungus is visible in the form of white cottony mycelium in the surface within which brown spherical sclerotic bark 1mm in diameter form.

CHAPTER - 5

ENVIRONMENTAL STUDIES



ENVIRONMENTAL STUDIES

"Because environmental factor are inte-related and dynamic and because they often exhibit delayed effects, an alteration of one factor frequently initiates a series of adjustment of for reaching and often unpredictable consequences".

— Daubenmine (1959)

The weather and soil conditions influence the seasonal development and geographical distribution of plant disease. The environment connotes all the external conditions affecting the life and development of an organism which include temperature, light and moisture and may also include living factors, such as competing micro-organism.

The environment can affect the development of plant disease in different ways. It can affect the perpetuation or over wintering of the pathogen from on growing to the next, the built-up of both primary and secondary inoculum, the dissemination of inoculum, germination and host penetration. The environment can affect growth and development of the host prior to being infected in such away as to affect its susceptibility. Finally, it can influence the actual development of disease after the host has become infected. Temperature and humidity in the

general atmosphere may be quite different than in the folior canopy of dense stands of growing plants.

Effect of Temperature on Mycoflora

Temperature is one of the most important environmental factor, which plays a significant role in covering various metabolic activities of the micro-organism. Morphological effects of temperature were observed on the conidia of *Cercospora sesami* (Chowdhury (1944) and on conidial fructification of *Aspergillus janus* (Raper and Thom. 1944).

According to Howker (1950) the optimum temperature for growth of fungi is usually between 20°C to 32°C and the cardinal points (minimum, optimum and maximum temperatures) for vegetative growth of the fungi are usually 0°C, 5°C, 20°C - 30°C and 30°C - 35°C, usually fungi do not grow below 0°C or above 40°C but exceptions are not infrequent. Panasonrko (1967) stated "As a rule fungi are more tolerance to lower then to higher temperatures since the latter coagulate cell proteins".

Tandon (1960) found that maximum and minimum temperatures for the growth of *Diplodia notatensis* were 38.8°C and 13.3°C respectively and optimum being 30°C. Bennet (1921) observed the mycelial growth of *Phoma apicola*

between 5°C and 28°C while Pycnidial development took place between 13.0°C and 26°C. Ames (1915) reported that the range for growth of *Monilia fructigena* and *Cephalothecium roseum* were 4°C - 30°C and 9°C - 35°C respectively while the corresponding ones for the production of conidia were 9°C - 30°C and 14°C - 30°C.

Massey (1926) observed that *Fusarium oxysporum* on Gladiali could grow over range of 5°C - 32°C with optimum temperature at 27.5°C. Agarwal (1955) reported that *Fusarium coeruleum* could not sporulate at 8°C but the sporulation was best at 20°C - 24°C and decreased at higher temperatures. Bhargava (1962) obtained best growth and sporulation of *Fusarium solani* at 25°C. According to Joffe and Palti (1972) in cultures isolates of *Fusarium solani* grew best at 24°C - 32°C. From the above mentioned facts it would be evident that before starting any physiological experiment, it is indispensal to have a thorough, knowledge about the temperature requirements of the organisms concerned.

Moreover, this would also give an idea about the environmental condition that would be most suitable for survival and propagation of a pathogen in nature. Therefore, it was considered necessary to determine the cardinal temperatures and especially the optimum temperature for growth and sporulation of the present

isolates. The following temperatures were take : 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C. The results are summarized in Table 4 to 11.

Alternaria radicina, A raphani and *A dauci* could grow between 9 range of 5°C - 35°C, poor growth of the 3 species of *Alternaria* was recorded of 5°C and *Cercospora beticola* and *C. carotae* could grow between 25°C - 32°C and *Fusarium oxysporum* and *Sclerotium rolfsii* grow between 25°C - 31°C and there poor growth of the 5°C. While high temperature i.e., 35°C supported moderate growth. Good growth of the mycoflora (*Alternaria, Botrytis, Cercospora, Fusarium* and *Sclerotium* species) was observed between 20°C - 31°C. Both growth and sporulation of the present fungi were at 25°C - 28°C.

Effect of Moisture Content on Seed Mycoflora

All types of seeds are subjected to mycobial contaminations under various physical and biological factors, including temperature, relative humidity, moisture content and duration of storage. The moisture content of seeds varied considerably with atmospheric humidity due to hygroscopic nature of seeds. Seeds rich in protein and starch, have higher moisture contents which results increase in respiration, water content and temperature of seeds causing loss of germination capacity, seed moisture encourages fungal growth (Hummel et al. 1954). There is a

minimum requirement of moisture content for the invasion and proliferation below which, deterioration is not possible. The water content of seed determines the fungal flora of seeds during storage and a slight variation in moisture content may change the flora both qualitatively as well as quantitatively. "In the range of moisture content between 14.0% and 15.0% in soyabeans or between 10.5% to 11.5% in flax seed, a difference of only 2% of moisture content makes a great difference in the rate of growth of storage fungi" as mentioned by Christensen and Kaufmann (1968).

Therefore, an attempt has been made to observe the effect of moisture content on percentage fungal infestation. The percentage infestation was observe at different ranges of moisture contents ranging from 6.5 - 7.5 to 13.6-14.5 percent. The percentage infestation was recorded within the range of 1 percent moisture content by blotter method. The results are recorded in Table 12.

A glance at Table 12 shows that the seeds of *Beta vulgaris, Daucus carota, Raphanus sativus, Brassica rapa* show infestation of fungal flora when kept above 7.5 moisture content while, the seeds escaped contamination with any type of fungal forms at lower (below 7.5) moisture content. Moisture range between 13.6-14.5 resulted maximum fungal infestation.

Moisture range between 6.5-7.5 show complete absence of any fungal form. Maximum (7.0%) and minimum (2.0%) infestation was noticed in *Daucus carota* respectively at lower moisture range (7.6-8.5%). Rest of the seed of vegetables including *Beta vulgaris, Daucus carota, Brasica rapa* and *Raphanus sativus* gave 51.32, 62.0, 72.0 and 60.0 percentage fungal infestation respectively, at 13.6-14.5 percentage, moisture content.

Table : 4

Effect of different temperatures and everage dry weight on sporulation of *Alternaria raphani*

Sl.No.	Temperature °C	Dry Weight (mg.)	Sporulation
1.	5	22.9	Poor
2.	10	30.5	Poor
3.	15	41.2	Fair
4.	20	49.8	Good
5.	25	56.4	Excellent
6.	30	51.7	Good
7.	35	16.9	Fair
8.	40	--	--

Table : 5

Effect of different temperatures and everage dry weight on sporulation of *Alternaria radicina*

Sl.No.	Temperature °C	Dry Weight (mg.)	Sporulation
1.	5	21.8	Poor
2.	10	27.6	Poor
3.	15	30.5	Fair
4.	20	40.2	Good
5.	25	45.8	Excellent
6.	30	50.2	Excellent
7.	35	53.9	Fair
8.	40	31.5	

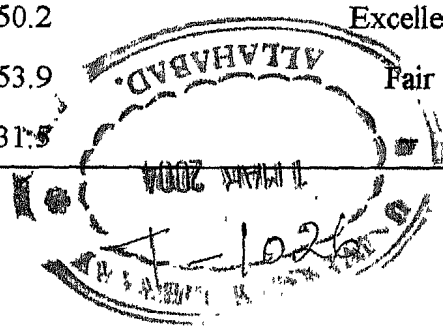


Table : 6

Effect of different temperatures and everage dry weight on sporulation of *Alternaria dauci*

Sl.No.	Temperature °C	Dry Weight (mg.)	Sporulation
1.	5	22.8	Poor
2.	10	26.4	Poor
3.	15	34.5	Fair
4.	20	40.2	Good
5.	25	48.8	Excellent
6.	30	54.2	Excellent
7.	35	56.2	Fair
8.	40	30.8	--

Table : 7

Effect of different temperatures and everage dry weight on sporulation of *Botrytis cinerea*

Sl.No.	Temperature °C	Dry Weight (mg.)	Sporulation
1.	5	--	--
2.	10	27.8	Poor
3.	15	30.8	Fair
4.	20	40.6	Good
5.	25	45.8	Excellent
6.	30	51.6	Good
7.	35	27.6	Fair
8.	40	--	--

Table : 8

Effect of different temperatures and everage dry weight on sporulatio of *Cercospora beticola*

Sl.No.	Temperature °C	Dry Weight (mg.)	Sporulation
1.	5	--	--
2.	10	27.6	Poor
3.	15	41.2	Fair
4.	20	50.2	Good
5.	25	52.6	Excellent
6.	30	54.8	Excellent
7.	35	29.6	Fair
8.	40	--	--

Table : 9

Effect of different temperatures and everage dry weight on sporulation of *Cercospora carotae*

Sl.No.	Temperature °C	Dry Weight (mg.)	Sporulation
1.	5	--	--
2.	10	27.7	Poor
3.	15	30.8	Fair
4.	20	40.1	Good
5.	25	45.2	Excellent
6.	30	50.8	Excellent
7.	35	30.6	Fair
8.	40	--	--

Table : 10

Effect of different temperatures and everage dry weight on sporulation of *Fusarium oxysporum*

Sl.No.	Temperature °C	Dry Weight (mg.)	Sporulation
1.	5	--	--
2.	10	27.9	Poor
3.	15	40.2	Fair
4.	20	53.8	Excellent
5.	25	59.8	Excellent
6.	30	54.9	Excellent
7.	35	31.5	Fair
8.	40	--	--

Table : 11

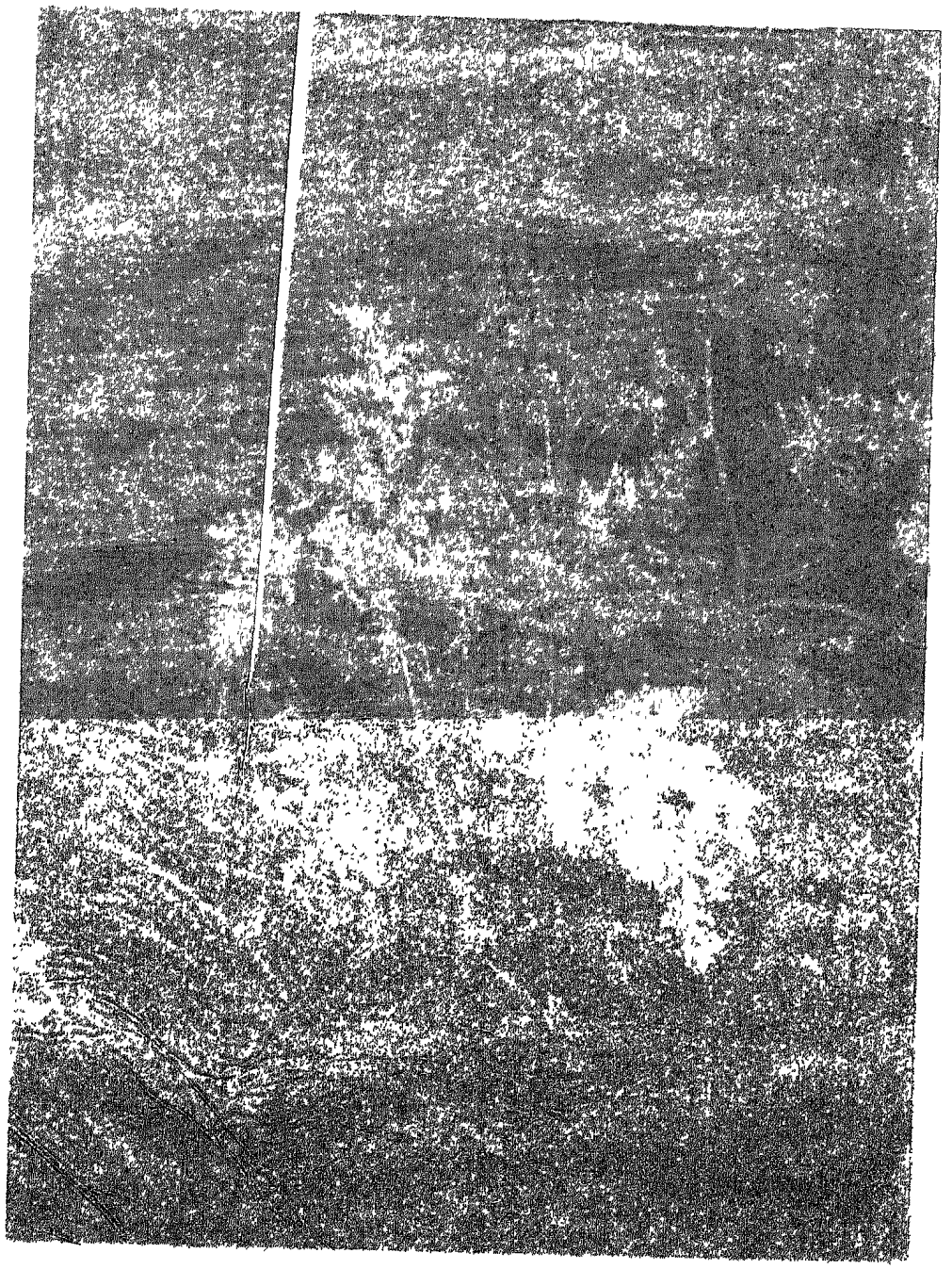
Effect of different temperatures and everage dry weight on sporulation of *Sclerotium rolfsii*

Sl.No.	Temperature °C	Dry Weight (mg.)	Sporulation
1.	5	--	--
2.	10	30.8	--
3.	15	35.9	Fair
4.	20	42.6	Good
5.	25	45.4	Excellent
6.	30	54.6	Excellent
7.	35	35.6	Poor
8.	40	--	--

Table : 12

Effect of moisture range on percentage infestation of fungal flora

Vegetables	Range of moisture percentage in different seeds of root vegetables							
	6.5-7.5	**7.6-8.5**	**8.6-9.5**	**9.6-10.5**	**10.6-11.5**	**11.6-12.5**	**12.6-13.5**	**13.6-14.5**
Beta vulgaris	00	3.40	5.12	8.00	17.10	28.00	40.00	51.32
Daucus carota	00	2.10	7.20	12.12	18.15	29.40	47.10	62.00
Raphanus sativus	00	4.24	15.00	22.22	25.00	32.14	35.20	72.00
Brassica rapa	00	4.10	10.20	14.32	16.00	22.00	28.00	60.00



STORAGE STUDIES

"People need food not production statistics and crop is not food until it is eaten. A programme to reduce storage losses probably could result in 10-12 percent increase in available food now in some developing countries, and might assure that whatever increase occur in production, in the future would be used for the nourishment to people, not for feeding pests"

— Christensen and Kaufmann (1969).

Seeds harbour fungi in storage that deteriorate their quality causing impairment of germination and production of abnormal seedlings. The season of sowing seeds varies from variety to variety and depends upon the atmospheric conditions. It is therefore, necessary to store the seeds for sowing and other purposes in future.

But the fungi in storage cause much damage to seeds in various way. Many parasitic fungi succeed due to poor storage condition. Some of the common storage fungi are the species of *Alternaria, Botrytis Cercospora, Sclerotium, Fusarium, Mucor* and *Penicillium.* Various species of *Alternaria, Cercospora, Fusarium, Botrytis* and *Sclerotium* are very common storage fungi encountered in seeds under storage.

The microbial infestation of seeds during storage responsible for biochemical, physical and physiological deterioration in seeds. The extent of deterioration depends upon the storage condition and storage period. Long duration of storage results poor health and loss of vigour of seeds as well as their viability. Hence, present investigation was aimed to study the effect of storage period on seed mycoflora.

The air-humidity during storage of seed is an essential for keeping the viability of seed. High humidity decreases the germination capacity while the number of abnormal seedlings increases.

Seeds are hydroscopic and once dried they can later gain moisture from seepage, leakage and moisture shifts, from production of moisture by insects, mites and fungi and from contact with air of high humidity. Here farmers dry seed when it is raining or the weather is hot and humid. The aim in drying grain then is not remove as much water as possible but to remove as little as possible to meet a given grade or to make the seed safe for storage for a given length of time, at a given temperature.

According to Papavizas and Christensen (1958) "Wheat with a moisture content upto 16% may be stored without obvious deterioration's for a year at a temperature of 10°C or below and wheat with a moisture content upto 18% may be

stored safely for as long as 19 months at temperature of 5°C". Qasem and Christensen (1958) working with sample of corn stored in the laboratory at moisture contents of 16% and 18% and temperature of 5°C, 10°C, 15°C, 20°C and 25°C. The Low temperature was as effective as low moisture content in preventing damage by the fungi tested.

In the present investigation an attempt has been made to isolate fungi from different seed stored for different length of time as well as stored in different containers. An attempt has also been made to isolate fungi from hand and machine threshed seeds. Effect of seed moisture on seed fungal flora has also been studied.

Different seed samples of different root vegetable (*Beta vulgaris, Daucus carota, Raphanus sativus* and *Brassica rapa*) stored for different length of time were collected from Market of Jaunpur, Allahabad city and adjacent village of Allahabad and Jaunpur city. Hand and machine threshed seed were collected from Bhidura, Holagarh, Dahiyawan, and Zapharabad villeges of Jaunpur and Allahabad district.

Fungi isolated from seeds of root vegetables stored at different length of time are summarized in Table - 13.

Effect of threshing and storage conditions on fungal contamination with seeds of root vegetable (*Beta vulgaris, Raphanus sativus, Brassica rapa* and *Daucus carota*) is recorded in Table - 14.

Percentage of infested seed due to various fungi at different range of moisture content of the root vegetables are recorded in Table - 12.

Fungi play an important role in causing damage to seeds by way to infection and bring about several undesirable changes, such as, reducing its germinating capacity and damaging its quality (Christensen and Lopez, 1963 ; Christensen and Kaufmann, 1965 and 1969 ; Tervet 1945 and Christensen and Mirocha, 1976).

Moisture content and temperature play a very important role in the development of storage fungi. High humidity and high temperature favour their development. Under low temperature and humidity, moisture content of the seed remain viable but inactive and as the temperature and humidity increases, the viable embryo becomes active and visible deterioration begins.

Storage fungi are present in the form of dormant mycelium outside or underneath the pericarp of the seed. Fungi associated with the storage seeds become active at the time of germination and cause heavy damage due to seed rot.

The economic value of seed is greatly influenced by the presence of associated mycoflora to the seed micro-environment. The storage fungi adversely affect the viability. The invasion of the embryos by storage fungi results in decreasing the germination power as suggested by Tuite and Christensen (1955), Qusem and Christensen (1958) and Papavizas and Christensen (1958).

Sometimes the discolouration of the embryo or the whole seed is brought about by storage fungi (Kenneth et al., 1947 ; Christensen, 1955; Papavizas and Christensen, 1958; Christensen and Linko, 1963 and Mehrotra, 1972) but if wheat grains with moisture content of 13% or above are kept long enough at a temperature of 35°C - 40°C the seeds turn brown even in the absence of storage fungi.

Fungal growth in stored seed is due to plenty of heat, which is due to storage fungi because bacteria can only grow at higher moisture content. Sometimes the temperature reaches over 90°C in the portion of bulk (Christensen and Kaufmann, 1968 and 1969) resulting decay of the seed. Rancidity is an important quality factor. The seeds after becoming rancid emits unpleasant taste or smell due to fungal invasion.

Among the metabolic products of fungi, the substances which bring about illness, abnormal physiological reactions to human beings and to warm blooded

animals are generally called mycotoxins. In recent years, it has been observed that mycotoxins produced in mouldy seed do not only inhibit the seed germination, but also have severe after effects on human beings on consumption, Mycotoxins are responsible for the reduction in seed germination. They cause wilting and stunting of seedlings and also affect the seed viability, as observed by Suryanarayana and Bhombe (1961) and Dwivedi and Tandon (1975).

A detailed information about the field and storage fungi associated with seeds of various vegetable crops are available (Kajansoon and Mathur, 1961 ; Harne and Nema, 1969 ; Sohi and Mahalay, 1974 ; Dwivedi and Tandon, 1976 ; Mc. Donald and Leach, 1976 ; Karwasra and Singh, 1982 ; Neseema et al., 1983 ; Deena and Basukachary, 1984 ; Gupta et al., 1984 ; Park and Kim, 1986 ; Prasad and Prasad 1987 and Dhyani et al., 1990) but only fragmentary information is available about the seed mycoflora of period but with very low frequency.

Results from Table - 13 clearly show that the variation in fungal flora is always noticed with the same type of seeds stored for different intervals of time. Storing seed for longer periods, i.e. above $2^{1/2}$ years, results in decline of fungal population. Fresh seed were associated with diverse type of field and storage fungi, which grew and multiply at the cost of the seed as long as the food and moisture supply was adequate and temperature was also not unfavourable.

It was observed that upto one year of storage, most of the seed samples were heavily invaded with both storage and field fungi. Later on, after the first year of production and upto 2 - 1/2 years of storage, there was an increase in the number of fungi in seed sample. But it gradually decreased after 3 years of storage.

Results from Table - 14 show that machine threshed seeds were found heavily infested with storage fungi, while hand threshed seeds indicated least infection. This is probably due to seed-coat injuries during storage seed storage in different containers, higher percentage of infection occurred in seed stored on the gummy bag. Tin container and sacks kept in wheat straw proved to be effective means of storage as a lasser percentage of seeds were found to be infested.

Table : 13

Percentage infestation of seeds of root vegetables stored for different length of time

Name of root vegetables	Percentage of infected seeds						
	Fresh seed	6 months	12 months	18 months	24 months	30 months	36 months
Beta vulgaris	15.0	17.00	22.00	18.00	20.00	26.67	22.33
Daucus carota	14.00	15.00	15.00	16.33	16.33	20.00	16.33
Raphanus sativus	13.67	14.67	16.00	15.67	17.33	21.67	16.67
Brassica rapa	17.33	25.00	24.00	21.67	17.00	13.00	14.00

Percentage infestation of seeds of root vegetables stored for diffrent length of time

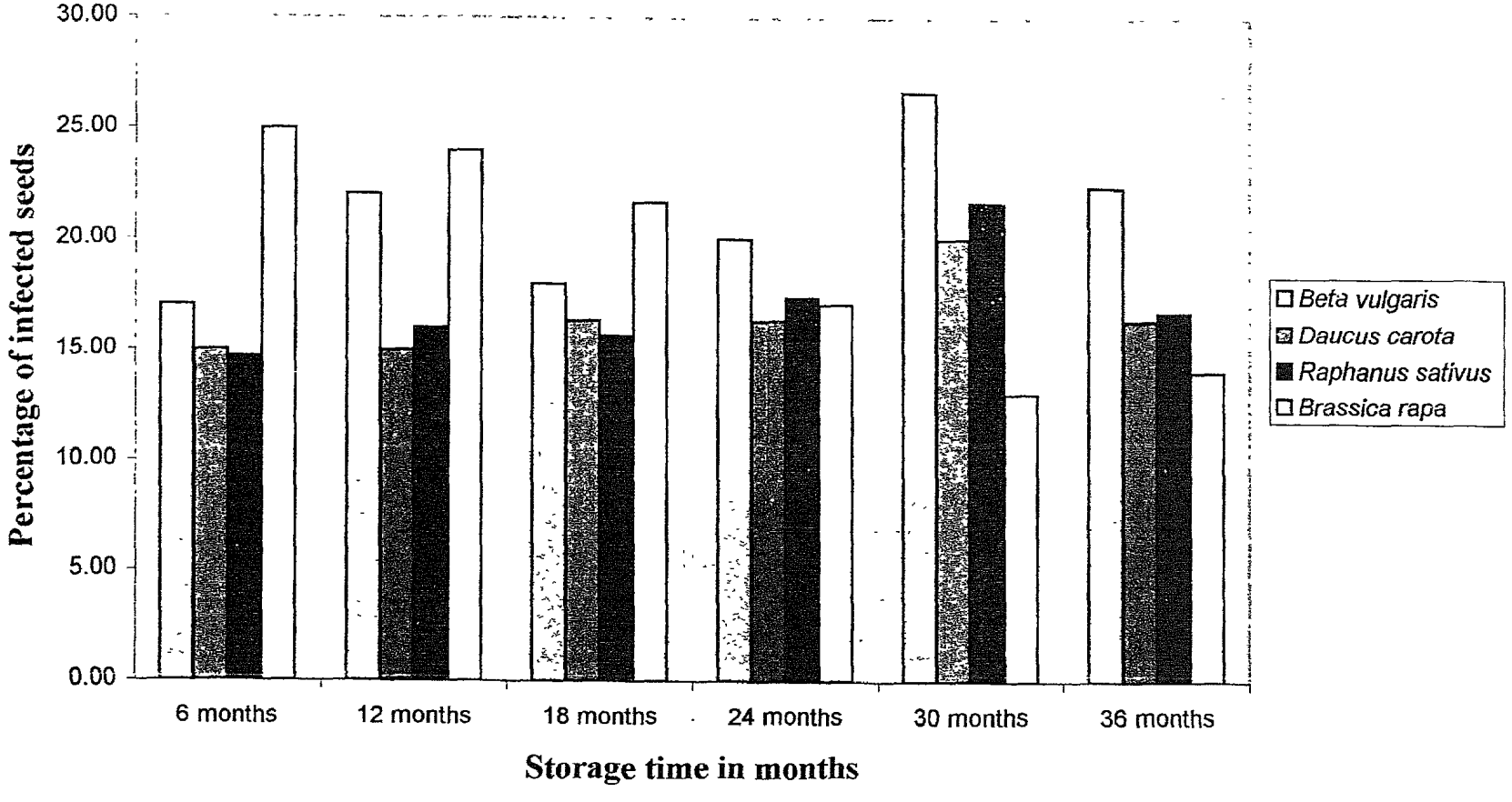


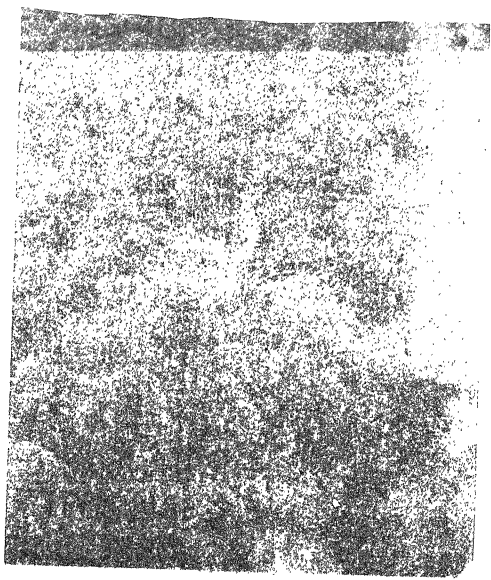
Table : 14

Showing effect of different threshing methods and storage conditions on fungal seed infestation of root vegetables

	Percentage of Seed infestation			
	Beet	**Carrot**	**Radish**	**Turnip**
Threshing Method				
1. Machine Threshed	25	29	26	24
2. Hand Threshed	20	25	23	22
Collection Sources				
1. Tin-container	12	17	15	14
2. Socks kept in wheat extra	11	15	13	12
2. In Gunny Bag	20	19	18	20

CHAPTER

SURVIVAL STORIES



SURVIVAL STUDIES

Seeds and soils are the resting places for a plant pathogen and it is a primary source of inoculum for onset of plant disease. Our knowledge regarding factors affecting survival of various wilt, blight, leaf spot and root-rot causing organism is however, very meagre. A number of seeds factors like temperature, moisture or amendment affect the population of pathogen and thus regulate the severity of infection. Creating unfavourable condition for the pathogen either by regulating the temperature and moisture or by amending the seed or soil with chemicals will help in controlling many diseases.

The present study deals with some factors affective survival of some pathogen i.e. *Alternaria, Fusarium, Cercospora, Sclerotium* and *Botrytis*, isolated from seeds and soils from different places. Though the effect of various microbiological factors on different seed fungi including ecology and pathogenicity have been studied by many investigators, yet a little attention have been paid towards the above aspect. However many workers including Stover (1953), Sen Gupta and Ray (1971), Prasad (1986), Rajpurohit (1982) and Singh (1987) have extensively reported the saprophytic behaviour of fungi in soil.

Temperature, humidity and other aspects of the physio-chemical conditions of the soil, the edaphic factors, particularly soil reaction, soil type and soil fertility are important environmental factors. In the period prior to infection these factors exercise influence on the predisposition of the host and the pre-paredness of the pathogens. If the environment favourable for the pathogen is the same as for the host, disease incidence may be very high, but it is also possible that the host thrives as such a temperature and humidity which is not favourable for the pathogen. This dissimilarity in suitabilities of environment for host and pathogen can be exploited for disease control. One of the method achieve this is to alter the date of sowing so that the susceptible stage of plant growth does not go inside with the environment that favours the pathogens.

In the present Agricultural experiment Farm of Allahabad University, Allahabad was selected for survival studies. The soil was dried and sieved and was infested with 3% maize meal sand culture. This was prepared in 250 ml conical flask. Rock flask was first filled up by 150 mg of sand and maize meal mixture (150 g. at dry clean sand+14.5 gm of maiz meal) and 20 ml of distilled water was carefully added (100 gm dry sand holds 20 ml water at saturation so 20 ml for 150 gm sand maize meal mixture gives about 65% saturation).

Such flask were taken autoclaved for 30 min at 20 lbs/inch^2 pressure and were inoculated with Agar inocular discs from a colony margin of a 7-10 days old culture of the fungus on Potato Dextrose Agar. Flasks were incubated for about 4 weeks at 25 ± 1°C and were shaken after 2 weeks to distribute the fungus.

When the above flasks containing 3% maize meal cultures of different root vegetables pathogen species were well grown they were then ready as inoculum for infesting the soil of the glass jar. 5 gm of the inoculum (maize meal sand culture) was mixed with 100 gm of unsterilized air dried soil. To assist in the distribution of inoculum the jars thoroughly shaken and 20 ml of water was added to give 50% saturation of soil. Such jars were weighed and weights were recorded on the jars. To maintain moisture content of 50% saturation all the jars were weighed on the pan-balance about twice a week and distilled water was added carefully until the original weight was restored. The inoculated glass jars were kept in the laboratory and covered by the Petri-dishes halves to reduce moisture loss. The small gaps served the purpose for air exchange. Five jars per treatment per fungal pathogen were taken. For the survival studies jars were shaken well before taking out the soil samples.

On the survival of the present species of fungus, the soil was infested with respective fungal species in the glass jars. They were incubated at a temperature

ranging from 5°C to 45°C after 7 days of incubation. Isolation were made on usual. The results are recorded in the Table - 15.

The maximum survival of all the *Alternaria species* (*A. raphani, A. dauci, A. radicina*) was observed between 25-30°C Prasad (1986). Their numbers (colonies) decreased as the temperature was either increased or decreased. Maximum number of colonies of *Alternaria* were observed at 28°C. The optimum a maximum temperature of survival *Botrytis cinerea* was between 20-27°C and cercospora 20 to 32°C. The maximum survival of *Fusarium oxysporum* was observed between 20-25°C and *Cercospora (C. carotae, C. beticola)* was observed between 27-30°C, Sen Gupta and Ray (1971) found the optimum maximum soil temperatures for competitive saprophytic survival of *Sclerotium rolfsii* was between 22-31°C. At 5°C and 40°C the survival of the present fungi was minimum. No colonies, however, could be recorded at 45°C.

Various investigators including Blair (1943) and Sen Gupta and Ray (1971) have reported the effect of soil moisture on the saprophytic activities of a number of fungi studied by them. In the present investigation the effect at soil moisture on the survival of different fungal species of *Alternaria, Botrytis, Cercospora, Fusarium* and *Sclerotium sp.*, pathogen was assayed in soil in which the water

holding capacity was adjusted at different percentage ranging from 10 to 100 percent the results are recorded in Table - 16.

The survival of the present fungal species was maximum when soil moisture was maintained at 30% water holding capacity. However above and below 30% water holding capacity of the soil, the survival of *Alternaria, Botrytis, Cercospora* and *Fusarium* declined and at 90-100% water holding capacity it was almost eliminated.

According to Sen Gupta and Ray (1971) in unsterilized soil maximum saprophytic activity of *sclerotium rolfsii* occurred at a wide range of relative low moisture (25-55% moisture holding capacity). Similarly Blair (1943) reported the effect of soil moisture on the saprophytic survival of *Rhizoctonia solani.* The reduction in population at high soil moisture was attributed by Blair (1943) to a decline in soil aeration with a increase in moisture content.

Table : 15

Effect of Temperature on the Survival of Fungal flora of root vegetables

Fungi	No. of colonies per 50 mg Soil								
	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C	45°C
Alternaria dauci	3	20	30	50	75	60	20	4	00
A. radicina	5	17	31	46	70	65	17	3	00
A. raphani	4	21	28	53	60	57	25	6	00
Botrytis cinerea	6	24	35	51	79	65	23	2	00
Cercospora carotae	5	26	40	50	71	55	21	3	00
C. beticola	4	30	45	56	75	70	20	4	00
Fusarium oxysporum	6	35	44	55	71	66	20	7	00
Sclerotium rolfsii	7	31	40	62	74	71	24	4	00

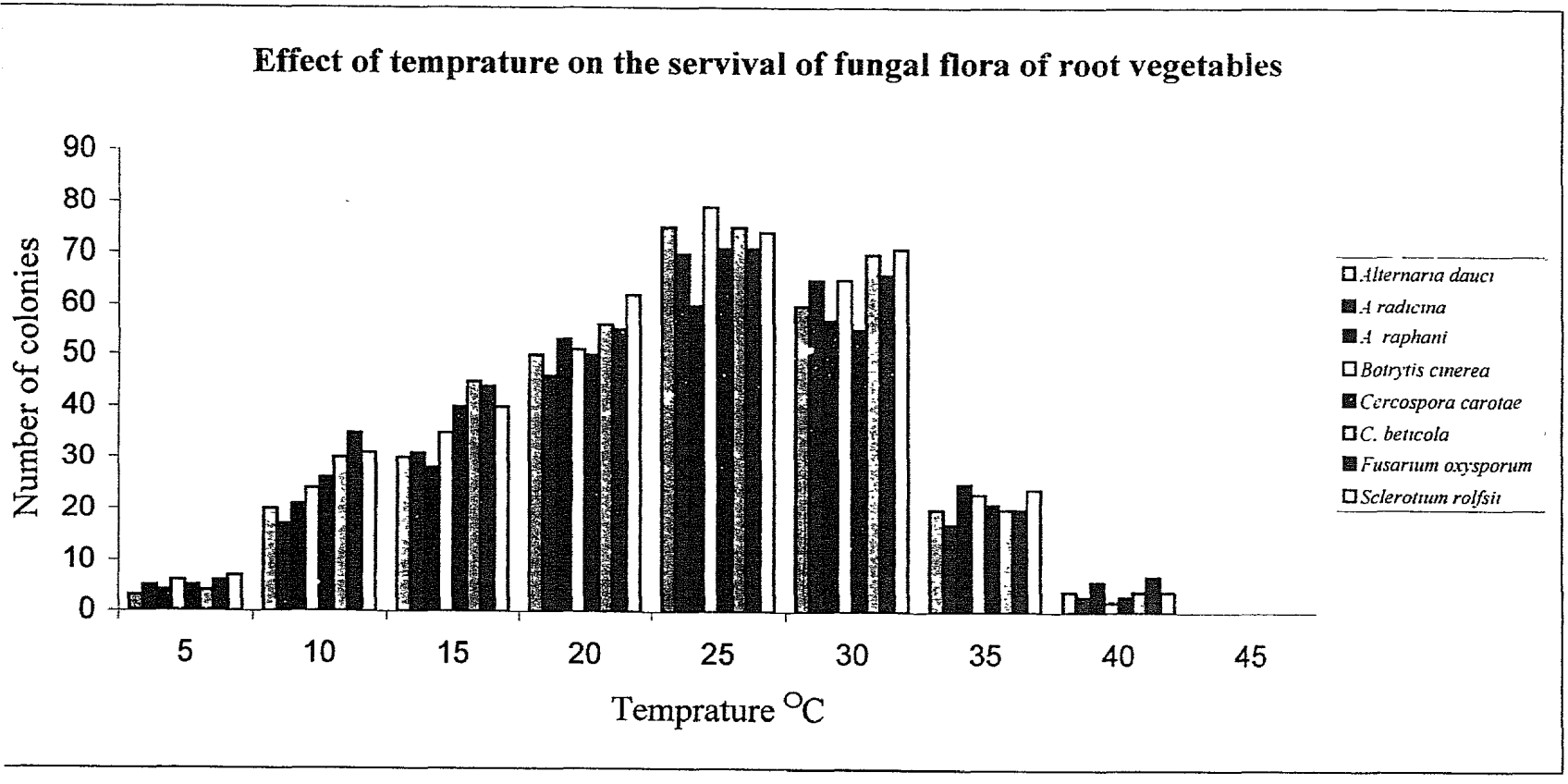
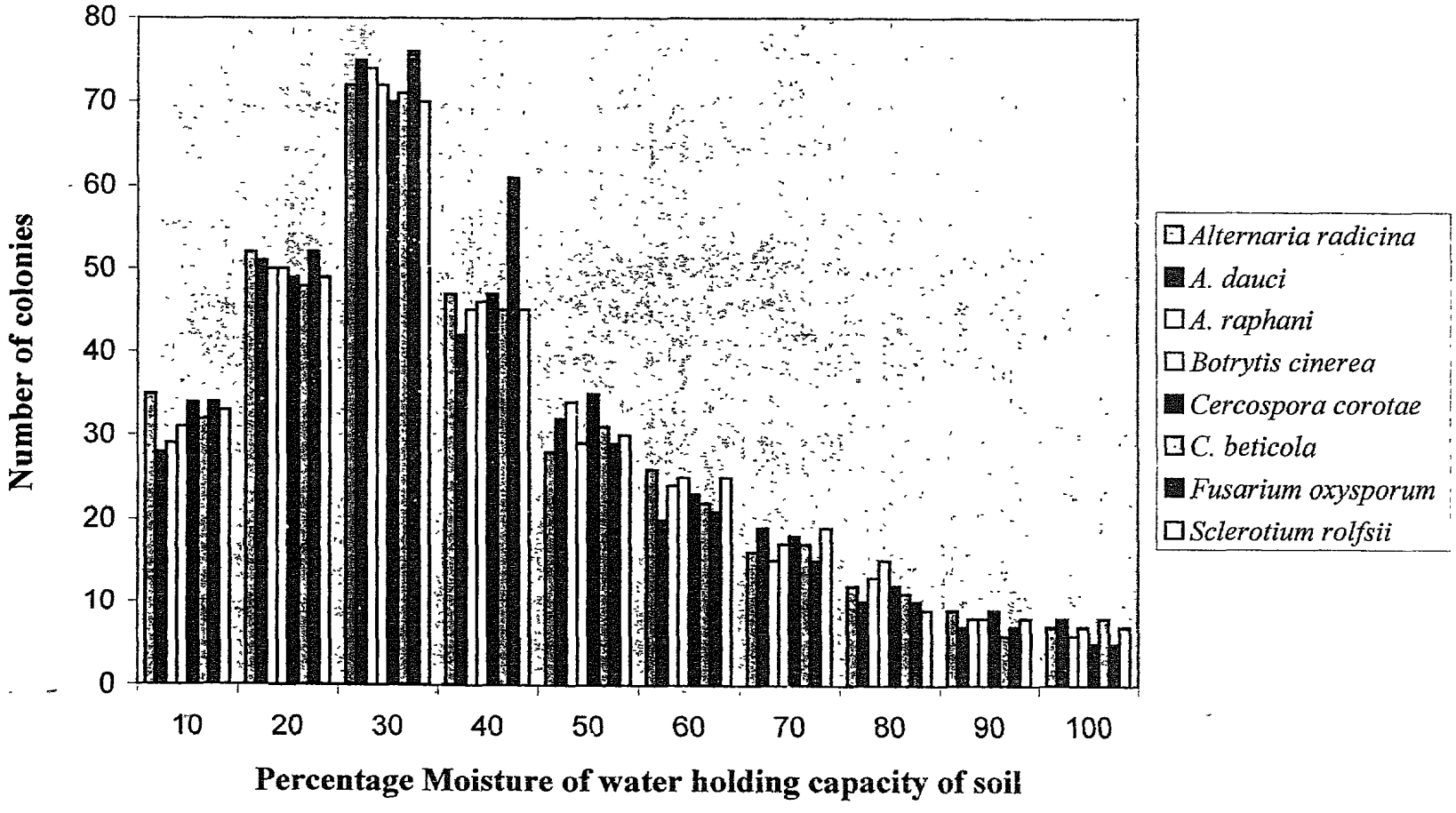

Effect of temprature on the servival of fungal flora of root vegetables
Number of colonies
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
35
40
45
Temprature °C
Alternaria dauci
A radicina
A raphani
Botrytis cinerea
Cercospora carotae
C. beticola
Fusarium oxysporum
Sclerotium rolfsii

Table : 16

Effect of Soil Moisture on the Survival of Fungal Flora

Fungi	Number of Colonies per 80 mg at Soil									
	% Moisture of water holding capacity of soil									
	10	20	30	40	50	60	70	80	90	100
Alternaria radicina	35	52	72	47	28	26	16	12	9	7
A. dauci	28	51	75	42	32	20	19	10	7	8
A. raphani	29	50	74	45	34	24	15	13	8	6
Botrytis cinerea	31	50	72	46	29	25	17	15	8	7
Cercospora corotae	34	49	70	47	35	23	18	12	9	5
C. beticola	32	48	71	45	31	22	17	11	6	8
Fusarium oxysporum	34	52	76	61	29	21	15	10	7	5
Sclerotium rolfsii	33	49	70	45	30	25	19	9	8	7

Effect of soil moisture on the survival of Fungal Flora



CHAPTER - 8

CONTROL STUDIES

CONTROL STUDIES

"When we know how the parasite breathes, feeds, exeretes and uses within its host and at its host expense, whatever bio-chemical process link it to that host, we may learn it to control it".

— Anonymous (1951)

To prevent Root vegetable diseases effectively, a knowledge of their nature and cause should be known. Once the causal agent has been correctly diagnosed then it is possible to prescribe effective methods for its control. However they may be prevented by a combination of plant protection principles that are necessary for effective protection against the several disease causing agencies.

The nature of diseases of root vegetables is seed as well as soil borne. Therefore major emphasis for the control of these diseases remained through resistant varieties.

In India, the first reference to plant protection is found in the oldest scriptures the Vedas (1500-500 B.C.). With the astounding growth of Chemistry, the knowledge about fungicides grew rapidly. Several chemicals were used in the

ancient time to control the diseases caused by fungi and other micro-organisms. The chemotherapeutants function both as protectant and as eradicant.

It is proved by various investigations that the fungicides were specific in action to pathogens as it had been seen that fungicide might be toxic to a particular fungus and at the same time be harmless to other fungi. According to Lilly and Barnett (1951) "There is no universal fungicide." Therefore, it became necessary to test the efficiency of some chemotherapeutants on the organisms under study. In the present investigation, following chemotherapeutants were evaluated in the laboratory. **Fungicides** : Bavistin, Captan, Ceresan, Difolatan, Dithane M-45, Dithane Z-78, Vitavax, Aurerofungin, Benlate, Brassicol, Cercobin, Ferbam, Plantvax, Thiram, Aretan and Agrosan GN.

The word fungicide has orginated from two latin words viz. fungus and caedo. The words caedo means "To kill". Thus a fungicide would be any agency which has the ability to kill the fungus. Many workers including Valaskova (1962), Sen Gupta and Roy (1971), Wells (1972), Byrde (1977) and others have tested a number of fungicide and made out standing contributions in this field.

Since the pathogen is a soil inhabitant and can survive in the soil for a long time. In a preliminary test the efficiency of a number of fungicide viz., Aureofungin (a helptaene antibiotic), Benlate (Methyl-1-Butylcarbamoyl-2-

benzemidazolecarbamate), Brassicol (Penta-chloronitrobenzene) cercobin (Benzene thiophanate) Difolatan [N-(1, 1, 2, 2 - tetrachloro ethyl, Sulfenyl) - CIS - 4-cylo-Hexene-1, 2-dicarboximide)] Ferbam (Ferric dimethyl dithio carbamate) Plantavax (DCMOD 2.3, dihydro-5-carboxanilido-6-methyl-1-4-oxathin-4, 4 dioxide) Thiram (Tetramythyle thiuram disulphide and Vitavax (2,3-dihydro-5-carboxamilido-6-methyl-1, 4-oxathin) and Tecto-40 (Thiabendazole (42.8%), 2-(4-Thiazolyl) benzimidazole) was also tested.

The fungicides were evaluated on the basis of their inhibitory effect on the vegetative hyphae by the method of suggested by Forsberg (1949). Small pieces of sterilized cotton threads (about 1.5 cm in length) were placed in petridishes containing Asthana and Howker's medium 'A' and were threads which got covered with vegetative hyphae of the various fungi within a week were subsequently rolled or dipped in the fungicide. The treated threads were transferred to another set of Petri-dishes containing above medium and were incubated at 25 ± 1°C for 7-10 days. Those fungicide which did not allow pathogen to grow were considered effective against a particular fungus.

The result shows in Table - 17, that benlate at 50 ppm, cercobin at 500 ppm, difolatan 100 ppm, plantvax at 500 ppm and vitavax at 1000 ppm, were found to be inhibitory to the growth of present organisms. Whereas, rest the

fungicide including the antibiotics and Aureofungin failed to inhibit the growth of present fungal species even at the concentration of 1000 ppm (maximum concentration taken). The concentrations of fungicides which inhibit the growth of the organism were then employed to check the population of fungul species of root vegetable in soil.

All the fungicides were amended in the soil at a concentrations which was found to be inhibitory to the growth of the organisms in culture media. Tecto-40 was amended to the soil at a concentration of 500 ppm. The fungal inoculum was incorporated in the soil after 24 hours of addition of the fungicides. Benlate and Difolatan were inhibitory at lower concentration to all the plant pathogens of root vegetables. Difolatan completely check the fungal population of root vegetable from the soil.

A seed-borne pathogen has greater capacity for spreading into growing crop than pathogens other than seed-borne. As the primary infection comes from the infected seeds experiment were carried out to control seed borne fungi by various fungicides. Several workers Anahosur et al. (1973) Chohan and Singh (1973), Daftari and Verma (1973), Jhamaria et al. (1975), Uyas and Nene (1975) Balkan et al. (1976) Tu and Cheng (1976), Prasad and Singh (1983), Rai and Singh (1986).

Gupta (1984), Mali and Jain (1985), Shivpuri (1988), Singh and Bhomik (1985), Sinha (1989), Ungaro (1984), Vishwakarma (1990), Yadav (1989).

On the basis of preliminary study eight fungicides viz. Bavistin, Captan, Ceresan dry, Agrosan GN, Thiram, Vitavax, Dithane M-45 and Dithane Z-78 were found effective. They were further tried on seeds of *Beta vulgaris, Daucus carota, Raphanus sativus* and *Brassica rapa*. There concentrations viz., 500, 750 and 1000 ppm solution were prepared with sterilized distilled water in sterilized flask and seed were treated with each concentrations.

Flask containing seed were shaken well and kept for a few hour. Treated seeds were placed in Petri-dishes containing PDA and incubated at 25 ± 1°C for a week to allow the growth of the fungi. Suitable controls were made by treating the seeds with sterilized distilled water. In another set of experiment, treated seeds were placed on the sterilized moist blotting papers.

Surface sterilized apparently healthy seeds of *Beta vulgaris, Daucus carota, Raphanus sativus* and *Brassica rapa* were treated with 500, 750 and 1000 ppm concentration of Ceresan, Agrosan GN, Thiram and Vitavax, they were the sown in post containing soil earlier infected with species of *Alternaria, Botrytis, Cercospora, Fusarium* and *Sclerotium*. Results are recorded in Table - 18.

Results from Table 19 to 22 clearly show that all the effective fungicides used for seed treatment pathogen, reduce the fungal contamination as compared to untreated seeds (control). If was further noticed that use of these fungicides on field does not cause any harmfull effect on their germinability.

Results from Table 18 clearly indicate that fungicide tried viz. Ceresan dry, Agrosan GN, Thiram and Vitavax used for seed treatment of root vegetables viz., *Beta vulgaris, Daucus carota, Raphanus sativus* and *Brassica rapa* against *Alternaria raphani, A. radicina, A. dauci, Botrytis cinerea, Cercospora beticola, C. carotae, Fusarium oxysporum* and *Sclerotium rolfsii* were very effective in checking various diseases. Ceresan was found to be more effective than others.

Effect of fungicidal amendment in soil mycoflora of root vegetable

Fungicides	Concentration in ppm	A	B	C	D	E	F	G	H
Benlate	50	3	4	6	4	5	4	3	4
	100	1	2	3	1	1	1	1	1
	500	--	--	--	--	--	--	--	--
	1000	--	--	--	--	--	--	--	--
Cercobin	50	40	22	24	20	15	16	7	10
	100	35	10	12	14	10	10	5	8
	500	04	07	05	05	10	10	04	04
	1000	00	02	02	01	05	--	--	--
Difolatan	50	5	7	3	2	1	1	2	4
	100	--	--	--	--	--	--	--	--
	500	--	--	--	--	--	--	--	--
	1000	--	--	--	--	--	--	--	--
Plantvax	50	20	15	14	20	24	20	15	12
	100	15	12	12	14	16	17	8	8
	500	05	04	--	--	-4	04	03	04
	1000	01	01	--	--	--	--	--	--
Tecto-40	50	12	15	06	15	17	10	16	12
	100	05	06	02	04	05	04	02	05
	500	--	--	--	--	--	--	--	--
	1000	--	--	--	--	--	--	--	--
Vetavax	50	30	25	29	29	30	25	22	26
	100	20	18	22	20	21	12	15	14
	500	05	06	02	02	04	02	00	05
	1000	--	--	--	--	--	--	--	--
Control	--	80	75	70	65	70	73	69	72

A- *Alternaria dauci* B- *A. radicina* C- *A. raphani* D- *Botrytis cinerea* E- *Cercospora beticola,*
F- *Cercospora carotae* G- *Fusariam oxysporum* H- *Sclerotium rolfsii*

Table : 18

Showing effect of fungicidal seed treatment on survival of seedling of root vegetables

Treatments	Concentration (ppm)	Percentage servival of seedling grown in infested soil with seed borne pathogens			
		A	B	C	D
Cereson dry	500	86	80	94	94
	750	98	94	98	94
	1000	98	94	98	96
Agrosan GN	500	60	64	48	74
	750	90	80	64	96
	1000	90	90	96	96
Thiram	500	72	86	84	90
	750	80	98	90	92
	1000	84	98	90	96
Vitavax	500	98	66	64	76
	750	88	94	86	88
	1000	94	94	86	92
Control		43	42	36	44

A-*Beta vulgaris*, B-*Daucus carota*, C-*Raphanus sativus*, D-*Brassica rapa*

Table : 19

Effect of different fungicides on fungal flora associated with (*Beta vulgaris*) seeds

Treatments	Concentration (ppm)	Percentage of seeds showing infestation after treatment with fungicides			
		A	B	C	D
Bavistin	500	03	01	03	02
	750	01	--	01	01
	1000	01	--	--	--
Ceresan dry	500	--	01	--	--
	750	--	--	--	--
	1000	--	--	--	--
Agrosan GN	500	02	01	03	01
	750	01	--	01	01
	1000	--	--	--	--
Captan	500	--	--	--	--
	750	--	--	--	--
	1000	--	--	--	
Dithane M-45	500	--	--	--	--
	750	--	--	--	--
	1000	--	--	--	--
Dithane Z-78	500	--	01	--	--
	750	--	--	--	--
	1000	--	--	--	--
Thiram	500	--	--	--	--
	750	--	--	--	--
	1000	--	--	--	--
Vitavax	500	04	03	02	01
	750	02	02	01	--
	1000	01	--	--	--
Control		12	03	08	10

A-*Botrytis cinerea*, B-*Cercospora beticola*, C-*Fusarium oxysporum*, D-*Sclerotium rolfsii*

Effect of different fungicides on fungal flora associated with (*Daucus catora*) seeds

Treatments	Concentration (ppm)	Percentage of seeds showing infestation after treatment with fungicides					
		A	B	C	D	E	F
Bavistin	500	--	--	--	03	01	--
	750	--	--	--	--	02	--
	1000	--	--	--	--	--	--
Ceresan dry	500	--	--	--	--	02	--
	750	--	--	--	--	--	--
	1000	--	--	--	--	--	--
Agrosan GN	500	5	3	2	--	1	1
	750	2	1	1	--	--	--
	1000	--	--	--	--	--	--
Captan	500	--	02	--	--	--	--
	750	--	--	--	--	--	--
	1000	--	--	--	--	--	--
Dithane M-45	500	--	01	--	--	--	--
	750	--	--	--	--	--	--
	1000	--	--	--	--	--	--
Dithane Z-78	500	--	01	--	--	--	--
	750	--	--	--	--	--	--
	1000	--	--	--	--	--	--
Thiram	500	02	--	--	--	--	--
	750	--	--	--	--	--	--
	1000	--	--	--	--	--	--
Vitavax	500	03	02	03	04	01	01
	750	01	01	01	02	--	--
	1000	--	--	--	--	--	--
Control		11	03	12	27	08	06

A-*Alternaria dauci*, B-*Alternaria radicina*, C-*Botrytis cinerea*, D-*Cercospora carotae*, E-*Fusarium oxysporum*, F-*Scleratium rolfsii*

Table : 21

Effect of different fungicides on fungal flora associated with (*Raphanus sativus)* seeds

Treatments	Concentration (ppm)	Percentage of seeds showing infestation after treatment with fungicides			
		A	B	C	D
Bavistin	500	05	04	02	04
	750	02	01	01	02
	1000	--	--	--	--
Cereson dry	500	--	--	--	--
	750	--	--	--	--
	1000	--	--	--	--
Agrosan GN	500	02	03	01	03
	750	01	01	01	01
	1000	--	--	--	--
Captan	500	04	03	02	03
	750	02	01	01	01
	1000	--	--	--	--
Dithane M-45	500	04	03	01	01
	750	02	--	--	--
	1000	--	--	--	--
Dithane Z-78	500	04	02	03	01
	750	02	01	01	--
	1000	--	--	--	--
Thiram	500	05	07	04	02
	750	02	04	02	01
	1000	--	--	--	--
Vitavax	500	03	04	02	01
	750	01	02	01	--
	1000	--	--	--	--
Control		03	02	06	04

A-*Alternaria raphani*, B-*Botrytis cinerea*, C-*Fusarium oxysporum*, D-*Sclerotium rolfsii*

Table : 22

Effect of different fungicides on fungal flora associated with (*Brassica rapa*) seeds

Treatments	Concentration (ppm)	Percentage of seeds showing infestation after treatment with fungicides			
		A	B	C	D
Bavistin	500	06	--	--	06
	750	02	--	--	--
	1000	01	--	--	--
Cereson dry	500	01	--	--	--
	750	02	--	04	--
	1000	--	--	--	--
Agrosan GN	500	2	--	4	--
	750	--	--	--	--
	1000	--	--	--	--
Captan	500	04	03	01	02
	750	02	01	--	--
	1000	--	--	--	--
Dithane M-45	500	--	02	--	--
	750	--	--	--	--
	1000	--	--	--	--
Dithane Z-78	500	05	10	05	02
	750	02	04	01	01
	1000	--	--	--	--
Thiram	500	02	05	02	03
	750	--	01	--	--
	1000	--	--	--	--
Vitavax	500	04	03	02	01
	750	02	01	01	01
	1000	--	--	--	--
Control		03	02	06	04

A-*Alternaria raphani*, B-*Botrytis cinerea*, C-*Fusarium oxysporum*, D-*Sclerotium rolfsii*

BIOLOGICAL CONTROL

"Any condition under which, or practice whereby survival or activity of a pathogen is reduced through the agency of any other living organism (except man himself), with the result that there is a reduction in the incidence of the disease caused by the pathogen."

— Garrett (1965)

The biological control of plant diseases has recently become an area of intensive research in view of the hazardous impact of pesticides and other agro-chemicals on the ecosystem. Amongst the bio-control agents, the mycoparasites have attained a significant position. Boosalis and Mankau (1965) suggested that efforts should be made to investigate the biological control of plant diseases through parasitism an plant pathologists should not lose the enthusiasm of searching new mycoparasites, because the greater the number of examples, the greater would be the chance of exploiting them as agents for biological control. For this, attempt should be made for their discovery, culture, ecology and successful application in natural habit.

Studies to determine the fungistatic or fungicidal nature of plant leaf-extracts have been made by Pandey et al. (1983) : A plant leaf-extract may exhibit wide range of fungitoxicity, Pandey et al. (1983), Tripathi et al. (1983).

Shekhawat and Prasad (1971); reported that out of 41 plant species tested, leaves of 13 and flowers of 4 plants contained antifungal activity while roots, fruits, pods and stems were rarely active.

Checked the *Fusarium lini* and *Fusarium solani* infection of flax and *Phaseolus valgaris* seeds by applying the onion and garlic extracts used in seed treatment and to infected soil. Shukla (2000).

A large number of plants have been reported in Ayurvedic literature dating back 2500-600 B.C. to possess medicinal properties. Mostly aqueous leaf-extracts of plants have been used to evaluate their fungitoxicity Janardhanan et al. (1963).

Leaf extracts of 15 medicinal plants were tested against fungal infestation of seeds of root vegetables. The method for obtaining the leaf extracts of these medicinal plants has been described in Table 23.

Fungal spores are known to be more sensitive to environment than mycelium, hence it was considered necessary to investigate the effect of leaf extracts of some medicinal plants on the germination of spores of present fungi.

To study the effect of these leaf-extracts on spore germination of *Alternaria radicina, A. raphani, A. dauci, Botrytis cinerea, Cercospora carotae, C. beticola, Fusarium oxysporum* and *Sclerotium rolfsii,* the filtrates were centrifuged for 1/2 hour at 2000 rpm. The extracts were dilute to 50%, 75% and 100%, Hoffman's (1860) method was followed for the study of spore germination. Results on the spore germination were recorded after 24 hours of the treatment and are presented in Table - 24.

A new concept for controlling seed-borne and soil-borne pathogen by leaf-extracts of some medicinal plants yielded satisfactory results in the present study. Complete control was observed in case of fungal seed contamination of *Beta vulgaris, Daucus carota, Raphanus sativus* and *Brassica rapa* when treated with leaf extracts of *Azadirachta indica, Ocimum sanctum, Strychnos nux-vomica, Allium cepa* and *Allium sativum.*

Seeds treated with an extract of *Azadirachta indica* developed a slight bitter taste, while the seeds treated with *Ocimum sanctum* extract improved in taste.

It is evident from Table - 24, that out of the 5 medicinal plant tried, leaf-extracts of *Strychnos nux-vomica, Allium cepa, Azadirachta indica, Ocium sanctum* and *Allium sativum,* at all the concentrations completely inhibited the

spore germination all the pathogens of root vegetable viz. (*Alternaria, Botrytis, Cercospora, Fusarium* and *Sclerotium sp.*.

Kapoor et al. (1981) established that extracts from convolvulus pluricaulis and Evolvulus alsinoides were inhibitory to *Alternaria brassica, A. brassicola* and *Fusarium oxysporum.*

Germination of fungal spores is essentially a process during which the normal metabolic and physiological activity is restored after dormancy. According to Gattlieb (1964) germination is the process by which a spore is transformed from a dormant state of low metabolic activity to one of the high metabolic activity. Formation of the germ tube is the outward and visible sign that the metabolic changes is complete.

Spores are known to be more sensitive to environment than mycelium hence it was considered necessary to investigate the effect of leaf-extract of some medicinal plant on the germination to spores of present fungi at room temperature ($25 \pm 1^{\circ}C$). Ten days old culture at the different fungal pathogen of root vegetable have been used.

It is evident from the table that out of five medicinal plant tried leaf extract of Neem at 100% concentration consulting checked the spore germination of all

the pathogen of root vegetables, fungi while in 100% Garlic (*Allium sativum*) leaf extract, spore of (*Fusarium, Botrytis* and *Sclerotium*) root-rot disease could only germinate up to 10%. In comparison to the above leaf extracts spore germination of Alternaria radicina, A. raphani, A. dauci, Botrytis cinarea, Cercospora carotae, Cercospora beticola, Fusarium oxysporum and Sclerotium rolfsii was 85, 87, 82, 92, 95, 83, 90 and 91 respectively in control sets (distilled water).

Table : 23

List of plants used for antifungal properties

Sl.No.	Common Name	Botanical Name
1.	Chrysenthemum	*Chrysethemum indicum*
2.	Costor	*Ricinus communis*
3.	Crotons	*Codiaeum variegatum*
4.	Dhatura	*Datura stramonium*
5.	Eucalyptus	*Eucalyptus teligoni*
6.	Ficus	*Ficus bengalensis*
7.	Garlic	*Allium sativum*
8.	Ixora	*Ixora singaporersis*
9.	Jasmine	*Jasminum grandiflorum*
10.	Lantana	*Lantana comora*
11.	Neem	*Azadirachta indica*
12.	Onion	*Allium cepa var. aggregatum*
13.	Papaya	*Carica papaya*
14.	Parthenium	*Parthenium hysterophovus*
15.	Poison bulb	*Strycnnos nuxvomica L.*

Table : 24
Showing effect of leaf-extract of various plant on spore germination

Leaf-extract	Concentration	A	B	C	D	E	F	G	H
Strychnos nux-vomica L.	50	40	60	45	40	35	50	51	54
	75	30	45	25	32	20	27	26	35
	100	00	02	05	02	00	00	05	05
Allium capa	50	50	40	35	40	45	53	57	45
	75	30	30	20	20	27	35	36	30
	100	00	02	05	05	00	00	05	03
Azadirachta indica	50	04	05	10	20	10	05	10	15
	75	00	02	01	02	00	02	00	03
	100	00	00	00	00	00	00	00	00
Occimum sanctum	50	50	40	45	30	25	42	47	37
	75	30	25	30	15	12	30	20	17
	100	10	17	15	00	00	18	12	08
Allium sativum	50	05	05	10	07	15	10	15	09
	75	03	04	02	03	00	00	02	00
	100	00	00	00	00	00	00	00	00
Control		**85**	**87**	**82**	**92**	**95**	**83**	**90**	**91**

A- Alternaria *dauci* B- *A. radicina* C- *A. raphani* D- *Botrytis cinerea* E- *Cercospora beticola,*
F- *Cercospora carotae* G- *Fusariam oxysporum* H- *Sclerotium rolfsii*

RESISTANT VARIETIES

The knowledge obtained in laboratories becomes power in the field "The fungi is made to sit and bag".

— Horsfall (1957)

There is considerable literature available on the breeding and in heritance of disease resistance in plant. A list of papers published on the inheritance of disease resistance in plant upto 1934 has been given by Hansen (1934). The literature on the genetics of disease resistance in vegetable has been reviewed by Walker (1965) and on field crop by Ausemus (1943) and Dicson (1956). Inheritance of resistance to viraldiseases has been reviewed by Holmas (1954), resistance rust by Hooker (1967) and to nematode by Hare (1965). Some more recent view articles are those of Hooker and Saxena (1971) Roane (1972) on trends in breeding for disease resistance crop.

The use of disease resistant varieties for controlling plant diseases has been termed as the painless method because it does not cost the farmers anythings. The resistant defends itself against a potential pathogen by means of a number of physical and chemical characteristics of the plant or which are formed in the plant in response to infection. The physical characteristics act as mechanical barriers

which prevent the trances and spread of pathogen in plant. The chemical factors which are toxic to the pathogen inhibit its growth and activity in the plant.

Since Biffen's (1905) elucidation of the inheritance of the resistance in single Mendelian fashion, spectacular progress has been made in our understanding of the genetic aspects of parasitism and disease resistance. The mechanisms of variability that make the pathogens versatile in their behaviour and host range are now well known. Flor (1955) explained host parasite interaction in flax rust by assuming gene for generationship between rust reaction in the host and pathogenicity in the parasite. Link and Walker (1933) reported presence of protocatecuie acid and catechal in the dry pigmented scales of onion bulbs resistant to *Colletotrichum circinans*. Flor (1955) reported that resistant varieties of flax exerete hydrocyanic acid (HCN) in the rhizosphere.

Orton (1990) obtained resistant cotton variety from selection and multiplication of resistant individually. He observed that some cotton plants did not show wilting in the heavily infected crop. A vast majority of crop varieties can be attack by a single pathogen or many different kinds of pathogen can attack a single variety. Most plants are naturally resistant to many pathogen. During evaluation of plant life weak and disease susceptible individuals have been

progressively eliminated by nature and the plants which exist today are those having developed resistance to most pathogens in a particular geographic area.

In the present study high yielding varieties of *Beta vulgaris* (Crimson globe, Buse E, Detriot dark red, Ramanskaya and triplex), *Daucus carota* (Pusa kesar, M-29, Selection-1, Golden Hert Indian Long red), *Raphanus sativus* ((Jaunpur jiant Arka nisant, whit long, Pusa chetki, Champion) and *Brassica rapa* (Pusa Kanchan, Pusa sweti, Pusa suarnima, Early milon red top and Golden ball); have been tested against seed-borne pathogen.

Results from the these experiments clearly show that Table - 25, out of 20 varieties of root vegetables tested against *Alternaria radicina, A. dauci, A. raphani, Botrytis cinerea, Cercospora beticola, C. carotae, Fusarium oxysporum* and *Sclerotium rolfsii*. Crimson globe and Bush-E (*Beta vulgaris*), M-29 and Selection-1 (*Daucus carota*), Arkanisant and Pusa Chetaki (*Raphanus sativus*) and Pusa swarnima and Golden ball (*Brassica rapa*) were found resistant to the pathogen under investigation.

Table : 25
Varieties of root vegetables showing infection percentage

Crop		A	B	C	D	E	F	G	H
Beta vulgaris	Crimsom globe	00	00	00	00	00	00	00	00
	Bush E	00	00	00	00	00	00	00	00
	Detriot dark red	00	06	00	04	15	00	00	10
	Ramanskaya	05	00	06	10	05	00	10	00
	Triplex	04	02	00	06	10	00	04	00
Daucus carota	Pusa Kesar	00	06	02	00	00	05	06	00
	M.-29	00	00	00	00	00	00	00	00
	Selection-1	00	00	00	00	00	00	00	00
	Golden Hert	10	00	10	10	06	00	04	02
	Indian long red	15	00	10	05	05	10	00	01
Raphanus sativus	Jaunpur Jiant	00	02	02	00	02	00	06	02
	Arka Nishant	00	00	00	00	00	00	00	00
	Whit Lang	00	05	04	06	00	10	02	00
	Pusa Chetaki	00	00	00	00	00	00	00	00
	Champion	00	00	10	02	06	00	06	00

Brassica rapa	Pusa kanchan	00	06	02	00	02	06	06	06
	Pursa Sweti	00	04	04	00	06	04	00	04
	Pusa swarnima	00	00	00	00	00	00	00	00
	Early milon red top	00	00	00	10	00	01	10	00
	Golden ball	00	00	00	00	00	00	00	00

A- *Alternaria dauci* B- *A. radicina* C- *A. raphani* D- *Botrytis cinerea* E- *Cercospora beticola,*
F- *Cercospora carotae* G- *Fusariam oxysporum* H- *Sclerotium rolfsii*

CHAPTER - 9

DISCUSSION AND CONCLUSION

DISCUSSION AND CONCLUSION

"As society grows geometrically, its complexity grows geometrically and its constraints grow geometrically. Some times one wonders if society will concentrate itself one day into obvious like the dinosour. Agriculture and forestry are constrained like every thing else and as they go, so goes plant pathology".

— Horsfall and Cowling (1977)

The term of root vegetable is applied to the edible herbaceous plants or plant parts which are commonly used for culinary purpose. Root vegetables play an important role in human diet, supplying some of the things in which other food materials are lacking. Root vegetables are rich and comparatively cheaper sources of vitamins, minerals and protein.

In developing countries like India where the pressure of population on land is continuously increasing vegetable plays a significant role in supplying a balanced diet to the poor. In general for a balanced diet about 85 gram root vegetable, for per human, per day is a must.

In India roughly 25% of the annual harvest is lost before consumption because of climate favourable to deterioration and ignorance of the farmer and

lack of facilities necessary to reduce or prevent such losses. The principal causes of loss in quantity and quality of root vegetable stored seeds are rodents, insects, mites and fungi; (Griffiths et al. 1959). Fungi which cause diseases of root vegetables are present in soil and seeds of both cultivated area and some of them are well known as disease causing pathogen.

The studies on isolation of mycoflora from root vegetable seeds in the present investigation yielded some interesting results. In all fungal species viz. (*Alternaria, Botrytis, Cercospora, Fusarium* and *Sclerotium*) were recorded from root vegetable seeds. Seed collected at random from various places did not show much variation in their fungal flora, although their frequencies were different in each samples.

The fungal isolates obtained from the above studies were screened for pathogenicity and among the fungal flora isolated from different root vegetable seeds, three species of *Alternaria* and two species of *Cercospora* and a species each of *Botrytis, Fusarium* and *Sclerotium* was found to be pathogenic on their respective hosts. The nature of diseases observed were root-rot, wilt, leaf-spot blight, seed-rot and head-rot. Apart from the above species each of *Aspergillus* and one species of *Penicillium* were found pathogenic causing seed-rot in their respective host. The nature of diseases observed were root-rot, head-rot, seed-rot,

leaf-spot, seedling blight and wilt. Apart from the above species of *Aspergillus* and species of *Penicillium* were found pathogenic causing seed-rot in their respective host. Among the pathogenic *Alternaria radicina, A. dauci, A. raphani, Botrytis cinerea, Cercospora beticola, C. carotae, Fusarium oxysporum* and *Sclerotium rolfsii* were observed most frequently to cause a number of disease viz. root-rot, seed and seedling rot, wilt, leaf-spot and blight.

Fungi are present in soil of both cultivated and non-cultivated fields and some of them are well known as root-rot and wilt causing pathogens. In the present study isolations from different fields at Allahabad and its adjacent regions carrying root vegetables constantly yielded species of *Alternaria, Cercospora, Botrytis, Fusarium* and *Sclerotium* besides a number of other fungi. Since *Alternaria, Cercospora, Botrytis, Fusarium* and *Sclerotium* are well known as blight, root-rot, leaf-spot and wilt causing pathogens, ecological factors governing their distribution in three selected fields of Allahabad viz. - Allahabad Agriculture Institute, Naini, Allahabad, Allahabad University Farm and Botanical Garden, Department of Botany were studied. The soil sample of all the fields were found to be alluvial. It was observed, that soil of all field under study contain maximum number of pathogen during the month of September, October, November and February, the soil condition as well as climate condition were optimum and

comparatively during above period. The fluctuation in fungal sp. in the non-rhizosphere and rhizosphere region may be due to edaphic factors viz., soil temperature and moisture. (Alexander, 1977). An increase in the number of rhizosphere fungi is perhaps due to "rhizosphere effect (Rovatt and Katzalson 1961, Anique et al. 1982).

Studies on pathogenicity of plant pathogen indicate that *Alternaria radicina, A. dauci, A. raphani, Botrytis cinerea, Cercospora beticola, C. carotae, Fusarium oxysporum* and *Sclerotium rolfsii* was most pathogenic as it could cause high percentage of root-rot, leaf-spot and blight at the seedling stage.

Soil is a resting place for plant pathogen and it is a primary source of inoculum for onset of plant diseases. Our knowledge regarding factors affecting survival of various wilt, root-rot, leaf spot and blight causing organism is, however, very meager. A number of soil factors like temperature and moisture affect the population of pathogen and thus regulate the severity of infection. Creating unfavourable conditions for the pathogen either by regulating the temperature, soil moisture or by ammending the soil with chemicals will help in controlling many diseases.

In the present study it is found that fungal population increased during rainy season and decreases in summer. During winter season also fungal population

decreases due to the decrease in soil temperature and moisture. The population of micro-organism declines with an increase in depth of soil profile. Similar plea about the distribution of micro-organisms at various depth, and the rhizosphere effect increased with increase in soil depth. Soil borne fungal pathogen are influenced by soil water factor which is an important factor for better growth and survival of such fungi (Cook and Papedick (1970).

Experiment dealing with effect of fungicide in-vitro indicate that the effect of Benlate and Difolatan were inhibitory at lower concentrations to all the present species of plant pathogen of root vegetables. Difolatan at 100 ppm could almost completely eliminate the fungal population of plant pathogen of root vegetable from the soil.

Thus on the basis of results obtained it is possible to check the population of fungal species of *Alternaria, Cercospora, Botrytis, Fusarium* and *Sclerotium* in the soil as well as in controlling the leaf-spot, blight, root-rot and wilt of root vegetables crops at seedling stage.

Fungi are capable of infecting a wide variety of substances due to ubiquitous nature. Seeds are prone to a number of diseases caused by fungi. As for as nutritional requirement of fungi is considered, they utilize the stored substances

in the host by absorbing them directly or by absorbing them indirectly or by converting the complex forms into simpler ones before utilization.

The variation in the fungal population inside and outside the seed is due to the environmental conditions of seed production. Its method of processing and storage conditions.

Seed mycoflora includes both the forms which come in contact with them in the field as well as those forms which come in association of seeds during harvest and transportation. Some additional fungal forms get associated with the seeds when storage conditions are improper. Unfortunately, our country suffers great loss due to post harvest damage caused by micro-organisms, which not only reduce the market value of vegetables but also affect their seeds.

A number of species of *Alternaria* have been reported as seed pathogens of various crops. *A. radicina* was reported as a seed borne fungus by several workers including Park and Lim (1986), Singh and Singh (1986), Arora (1987), Dutt and Roy (1980), Mahajan and Mora (1989), Sinha and Prasad (1989) as well as Vishnupurikar and Godbole (1989) and Dhyani et al. (1990). Bhargava and Khare (1988), Groues and Skalko (1944).

Botrytis cinerea was also reported as seed borne fungus by Agarwal and Singh (1974), Dhyani et al. (1990) and *Chaetomium globosum* was reported by Swaroop and Mathur (1972), Singh and Singh (1986) as well as Pandey and Dwivedi (1987) as a seed-borne fungus.

Fungi were isolated from the seed sample of root vegetable viz. Beet (*Beta vulgaris*) Carrot, (*Dacus carota*) Radish (*Raphanus sativus)* and Turnip (*Brassica rapa*). In addition to their report a number of other fungi such as *Alternaria* (*A. radicina, A. dauci, A. raphani*) *Botrytis cinerea, Cercospora (C. beticola, C. carotae) Fusarium oxysporum* and *Sclerotium rolfsii* have reported to occur during the present studies (Bedi (1956).

Seeds of different root vegetable crops viz. Beet, Carrot, Radish and Turnip included in the present study showed heavy fungal infestation, they showed both external and internal association of fungi. However, internal association was limited to a smaller proportion of seeds while nearly hundred percent seeds showed external association.

The present investigation clearly indicates that the extent of mycoflora associate with the seeds varied with the nature of seed. These variations may either be due to the climatic conditions at the time of storage or physico-chemical nature of the seeds.

During present investigation the seeds of Turnip and Radish had low percentage infestation and lower germination percentage. The other seeds of Beet, Carrot, Radish and Turnip showed high germination percentage as well as high infestation of fungal forms. Therefore, it is concluded that extent of percentage infestation and the germinability of seeds showed no positive co-relation.

Tervet (1945), Wallace (1959), Ullstrup (1961), Fields and King (1962), Brooke and White (1966), Jones et al. (1972), Zimer and Zimmermann (1972) and Smedegard - Peterson (1974) pointed out the direct impact of field and storage fungi on seeds. These fungi effect the germination capacity of seeds either by reducing or by eliminating the germination power.

Fungi showed diverse mode of infection. Some fungi causing damage of the plant upto a wide extent while, other have restricted mode of infection. The fungi associated with seeds result pre and post-emergence rotting of the germinating seeds and the reduction in the vigour of the seedling (Jhamaria et al. 1975; Bilgrami et al. 1979 ; Singh and Singhal, 1983 and Arora 1987).

The fall in the germination of seeds may be due to secretion of toxin substances showing inhibitory responses on seed germination. (Asplin and Carnaghan, 1961 ; Krough et al. 1966 ; Diener and Davis, 1967; and Bilgrami et al. 1979). The present results revealed that most of the fungi under study reduced

the percentage seed germination. Besides pathogenic forms, some other forms also effect the seed germination and vigour of the seedling has been obtained by Dempsey and Chandler (1963) and Leelavathy (1969).

The rate at which seed deteriorated in storage was influenced by a number of factors, like moisture content, temperature, the kind of fungal flora involved, the length of storage period, type of storage containers, the rate at which the fungi grow, the condition of seed and the extent and perhaps the severity of injuries on the seed coat of the seeds.

Variation in fungal population in the same type of seed collected from different places and stored for different intervals of time is reported earlier by Bhargava and Shukla (1978). According to Christensen and Dorworth (1966), Christensen (1972) and Dhingra et al., (1973), moisture content and temperature are responsible for the mycoflora in the initial stage of storage is greater than in the later stage, when the condition becomes reverse. This is due to change in relative humidity.

The change in the seed mycoflora during storage has been investigated by a number of workers (Tervet 1945; Qusem and Christensen, 1958; Field and King, 1965; Burroughs and Sauer, 1971; Christensen, 1972; Dhingra et al. 1973 and Niles, 1976). They also reported increase in the population of storage fungi and

decrease in field fungi. The population of some of the common storage fungi such as *Alternaria, Botrytis, Cercospora, Fusarium* and *Sclerotium* decreases with the increases in storage period. Christensen and Lopez (1963) also reported similar results.

Christensen and Kaufmann (1965) noticed the decrease in seed germination with the increase in storage period. In the present investigation, it has also been observed that with the increase in storage period the seed germination decreased. On the basis of above results, it can be concluded that storage period play an important role in maintaining the longevity of stored seeds.

Temperature is an important environmental factor affecting the metabolic activities of the fungi. It is evident from the study of eight plant pathogen of root vegetable that normally grow between a range of 20°C and 30°C and when the poor growth is recorded at below 10°C and above 30°C.

Absence of fungal growth below 7.5 percent moisture content indicates that some initial moisture content is required for growth and proliferation of fungi. It is generally seen that high moisture content (12.5 - 13.5) favours high fungal infestation. Therefore, it is recommended that the seeds should be stored in aseptic conditions under controlled humidity in cool and dry places which would in no

way adversely affect the nutritional content of the vegetable seeds and safe the seeds from infestation by fungi.

Though much emphasis had been laid on suitability of the storage structures and bins, yet there was ample scope for their improvement. An ideal storage structure should necessarily be damp-proof, air tight resistant to heat and as far as possible it should be termite proof as well, thus the silo (above ground steel) had been recommended for bulk storage of seeds.

Cotton et al., (1953), stated that "The proper utilization of nature supplemented by artificial methods can be depended upon to conserve seed in storage for an indefinite period without material loss from insect attacks". The major types of losses caused by fungi growing in stored seeds were, decrease in germinability, discolouration various adverse biochemical changes, production of toxins and loss in weight. Fungi frequently found on seeds were in their imperfect state and only rarely in their perfect states. Cotton and Grey, (1948); emphasized "Good house keeping is the simplest and best preventive measures."

Storage studies revealed that the seed samples stored upto 12 months became heavily associated with both storage and field fungi and their population increased gradually upto 3 years of storage. But after 3 years of storage its population decline. It was further noticed that machine threshed seeds were more

amenable to fungal-flora than hand threshed seeds, possibly due to more injury caused in the former process, Kulik, (1973). Isolation from the seeds collected from various containers showed that the tin containers and the sacks kept in wheat straw has less contaminations.

The microbial infestation of seeds after harvest caused development of many physical; biochemical and physiological deterioration's in seed. The rate of deterioration increased with poor and improper storage conditions.

The seeds became contaiminated at or prior to harvest and so there was no reason to expect that the two adjacent seeds at planting would carry the same pathogen. In this manner, seed-borne diseases characteristically exhibited a random pattern of infected individuals throughout the field. Many seed-borne diseases could later have an air-borne phase of dispersal in the early stages of the epidemic.

After maturation of the seed, the infection was likely to occur during the curing of the seed-crop, while the harvested crop was placed in sacks, piles of stocks during the threshing and subsequent processing during storage. The entire process provided conditions favourable for spreading of pathogens, saprophytes and for establishment of infection.

The American phytopathological Society, (1943); defined a fungicide as "A chemical or phyical agent that kills, or inhabits, the development of fungus spores or mycelium".

Horsfall et al. (1940) ; were among the first to suggest that the protective value of fungicides in the field could be predicted on the basis of laboratory bioassay tests. Horsfall considered that fungitoxicity and tenacity were the two fundamental components of potential protective value against plant disease in the field. One purpose of chemical seed treatment was to inactivate surface-borne fungi that caused seed decay or seedling blights. Chemical seed treatment was used to create a protective zone around the seed through with soil-borne micro-organisms failed to penetrate. Appropriate treatment of seeds got rid of the seed-borne pathogens and control the diseases to a large extent that would have results otherwise.

Most protective fungicides had relatively short effective residual life and consequently were required to be reapplied to maintain residual protection of susceptible tissue. Seed-borne infection of *Fusarium semitectum* in soyabean was controlled by seed treatment, Saharan and Gupta (1947).

From the present investigation it is evident that there is a reduction in the fungal population without exerting any harmful effect on germination of seeds

after treating the seeds with suitable chemotherapeutants. The results are also supported by various workers including Nene et al. (1969). Grover and Bansal (1970), Kandian and Suryanarayan (1971), Dharamvir et al. (1972), Agarwal et al. (1974), Dwivedi and Tandon (1975), Jhamaria et al. (1975), Bolkman et al. (1976), Jharia et al. (1977), as well as Mishra and Dharmvir (1990) also reported that the seed germination increased after treating the seeds with various chemotherapeutants.

Adverse effect of fungicides of seed germination has also been reported by Rangaswami (1969), Swaminathan and Sullia (1969), Backman and Hamond (1976) and Bolkmen et al. (1976). All the chemotherapeutants used during present investigation could reduce mycobial population at higher concentration.

Seed treatment could be utilized to inactivate the superficial fungal flora by creating a protective zone around the seed thus the soil borne fungi become unable to penetrate the seed. The seeds treated with effective chemotherapeutants could be used for sowing purpose. Only the effective chemotherapeutant with high germination capacity of the seed be used. Fungicides used in the present studies for seed treatment included among others Bavistin, Ceresan, Agrosan GN, Thiram, Captan, Dithane M-45, Dithane Z-78 and Vitavax were proved to be very effective in inducing seed germination as well as reducing the mycoflora of the root

vegetables. In recent years several systematic fungicide have been developed and their use and effectiveness are being studied.

The present investigations clearly revealed that the seed mycoflora played an important role in bringing about the disease development in field. Therefore, it became essential to device suitable control measures for these maladies. The primary screeing of 16 fungicides were determined against the plant pathogen viz. (*Alternaria, Botrytis, Cercospora, Fusarium and Sclerotium*) species and among them 13 were found to be effective, as they retarded the mycobial growth. Fungicide were also tested against fungal infection and to control disease coming on seedling and plants. It was observed that fungicidal seed treatment reduced the fungal infestation without any harmful effect on seed germinability.

To save the germ and to chek the development of various disease caused by *Alternaria dauci, A. radicina, A. raphani, Botrytis cinerea, Cercospora carotae, C. beticola, Fusarium oxysporum* and *Sclerotium rolfsii* seeds of *Beta vulgaris, Daucus carota, Raphanus sativus* and *Brassica rapa* should be treated with any one fungicides viz. Agrosan, Ceresan, Vitavax and Thiram, at 1000 ppm. Ceresan was found to be more effective.

Biological control has been broadly defined as the encouragement of beneficial organism already existing in a locality or of the introduction of suitable

new species, exotic parasite organism, which are parasite on harmfull pathogens in a locality where the pest is thriving with a view to control the disease. Through the practical application of biological control is of comparatively a recent origin, interactions between different organism in an ecosystem must have been in existent for the maintenance of a stable population in an environment.

Further, a novel idea of controlling seed-borne disease by leaf extracts revealed that leaf-extracts of *Azadirachta indica, Ocimum sanctum, Allium sativum, Allium cepa* and *Strychnos nux-vomica* controlled fungal seed contamination. Leaf-extracts at the medicinal plant checked the spore germination of the fungi investigated. The possibility of the presence of different chemical compound, fungicidal or fungistatic in action in these extracts which exerted inhibitory influence upon germination of fungal spores was always there.

Leaf extracts of *Bougainvillea glabra* and *Piper betle* were inhibitory to *P. monospermum* Alice, (1984). Partial inhibition of *H. sativum* by leaf extracts of *Lowsonia inermis* was reported by Tripathi et al., (1978). Leaf extracts of *Eucalyptus brockwayi* and *E. occidentalis* incorporated in PDA medium restricted the mycelial growth of *Pestalotiopsis mangiferae.* Garlic extract inhibited the mycelial growth of *Gibberella zeae* at the concentration of 8000 and 10000 ppm.

Spore germination of *Drechslera, Fusarium oxysporum, Alternaria alternata* and *Corynespora cassicola* was inhibited by the aqueous extract of onion, garlic, *Parthenium histopum*, cotton and *Phaseolus altropurpurens*.

Plant breeders and plant pathologists in the past concentrated primarily to evolve varieties with high degree of resistance against specific pathogens or few races and biotypes of pathogens characterised by specific host-parasite interaction and this type of control is designated as "Vertical resistance". In contrast to vertical resistance, another type is met which is fairly non specific with moderate level of resistance against a large number of pathogens or races of pathogens is commonly known as field or horizontal resistance.

In breeding for disease resistance, the aim should be to develop such varieties that may have characters which may be mechanical or non specific chemical substances as inhibitors or can induce enzymatic reactions resulting in inhibition of pathogens or may induce specific host parasite reaction. Numerous examples of successful breeding of plants for plant disease-resistance are cited by Caldwell, (1966). Some authorities believe that this is the 'Ultimate' in plant disease control and the only approach worthy of serious scientific consideration.

Cultivars of *Cicer arietinum* resistant to *Ascochyta rabiei* had Malic acid in leaves, Hafiz, (1952); reported that pea selections with pigmented seed substances

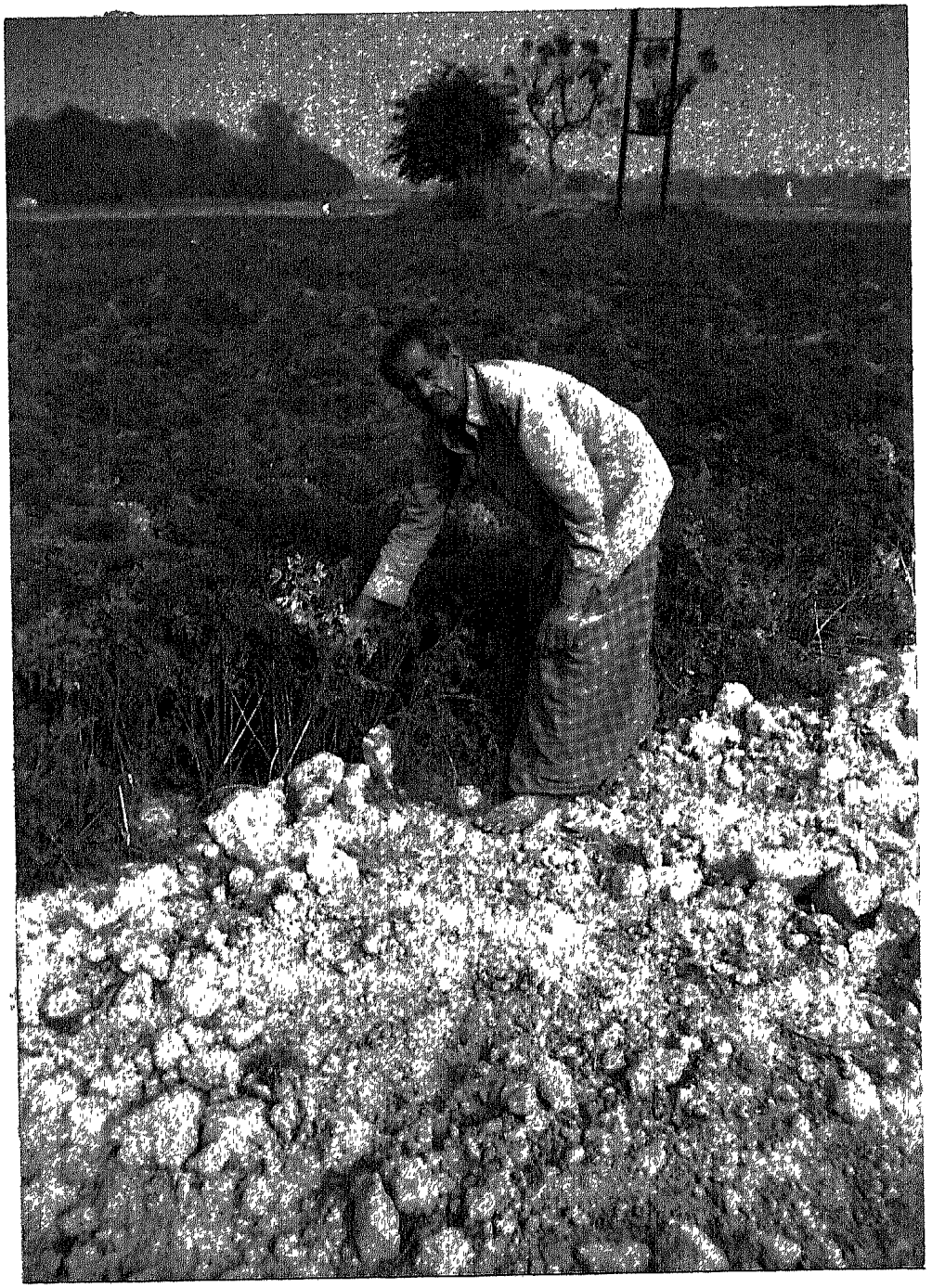
during germination which inhibited sporulation and conidial germination of *Fusarium solani, F. pisi*. A phytoalexin, Pisatin in *Pisum sativum* was reported to be a critical factor in resistance of pea to fungi parasitic in this host.

Studies on high yielding varieties revealed that out of 20 high yielding varieties of root vegetables crops tested, eight were found to be resistant to all the pathogens. Most of the field fungi were sensitive to high temperatures and usually disappeared under such temperature conditions Lutey and Christensen, (1963); *Alternaria alternata* and *Rhizopus stolonifer*, however, developed at temperatures above 40°C Hellberg and Holk, (1972).

Besides chemical control, there are certain preventive measures like seeds stored in air tight containers, and at 7.5% moisture content besides the and improvement in harvesting practices to prevent seed-coat damage have been also suggested for vegetable crop.

CHAPTER - 10

SUMMARY



SUMMARY

Enormous losses are sometimes sustained in root vegetables crops due to fungi. The earning can easily be doubled if adequate attention is paid to the improved agricultural practices.

A comprehensive survey of various markets and cultivated fields of root vegetables of Allahabad, Jaunpur, Pratapgarh, Varanasi and Sant Ravi Das Nagar districts of Uttar Pradesh was made in which these root vegetable i.e. *Beta vulgaris, Raphanus sativus, Daucus carota* and *Brassica rapa* were observed. From these surveys it was revealed that about 10 - 60 percent root vegetables were affected from fungal diseases and the nature of diseases were wilt, seed-rots, leaf-blights, leaf-spots and root-rots.

Isolations were made from the soil rhizosphere, rhizoplane, diseased roots, root lets and rotted tissue of infected crops. Fungi were isolated, purified and maintained on malt extract and Potato Dextrose Agar media for further examination and pathogenicity tests. Morphological studies were carried out and indentifications were made. The fungi isolated from the infected plant parts of root

vegetables, its treated as well as untreated seeds and from soil samples were as follows:-

Fungi isolated from seeds and infected plant parts of root vegetable

Vegetables	Untreated seed	Treated seed	Diseased plant parts
Beet (*Beta vulgaries*)	*Alternaria alternata*	*Alternaria sp.*	Leaf
	A. ricini		
	A. tencissima		
	Aspergillus flavus		
	A. fumigatus		
	A. niger		
	A. terreus		
	Botrytis cinerea	*Botrytis cinerea*	Root
	Cercospora beticola		
	Curvularia lunata		
	C. robusta		
	Fusarium equiseti	*Fusarium equiseti*	Root
	F. oxysporum	*F. oxysporum*	Root
	F. semitectum	*F. semitectum*	Root
	F. solani	*F. solani*	Root
	Helminthosporium spp.		
	Rhizoctonia spp.		
	Slcerotium rolfsii	*Slcerotium rolfsii*	Root
Carrot (*Daucus carota*)	*Alternaria alternata*	*Alternaria alternata*	Leaf
	A. dianthicola		
	A. raphani		
	A. radicina	*A. radicina*	Root & Leaf
	A. dauci	*A. dauci*	Leaf
	Aspergillus clavatus		
	A. fumigatus		
	A. niger		
	Botrytis cinerea	*Botrytis cinerea*	Root
	Cercospora carotae	*Cercospora carotae*	Leaf
	Curvularia andropogonis		
	C. lunata		
	C. robusta		
	Fusarium oxysporum	*Fusarium oxysporum*	Root
	F. roseum	*F. roseum*	Root

	F. solani	*F. solani*	Root
	Helminthosporium sp.	*Fusarium sp.*	Root
	Rhizopus stalonifer		
	Sclerotium rolfsii	*Sclerotium rolfsii*	Root
Radish (*Raphnus sativus*)	*Alternaria alternata*	*Alternaria alternata*	Leaf
	A. dianthiocola		
	A. raphani	*A. raphani*	Leaf
	A. brassicae	*A. brassicae*	Leaf
	Aspergillus clavatus		
	A. flavus		
	A. niger		
	Botrytis cinerea		
	Curvularia lunata		
	C. robusta		
	Fusarium oxysporum	*Fusarium oxysporum*	Root
	F. roseum	*F. roseum*	Root
	Helminthosporium sp.	*Fusarium solani*	Root
	Mucor mucedo	*Fusarium sp.*	Root
	Rhizoctonia solani		
	Sclerotium rolfsii		
Turnip (*Brassica rapa*)	*Alternaria alternata*	*Alternaria alternata*	Leaf
	A. dianthiocola		
	A. brassicicola		
	A. brassicae		
	A. raphani	*A. raphani*	Leaf
	Aspergillus clavatus		
	A. flavus		
	A. niger		
	Botrytis cinerea	*Botrytis cinerea*	Root
	Curvularia andropogonis		
	C. robusta		
	Fusarium oxysporum	*Fusarium oxysporum*	Root
	F. solani	*F. solani*	Root
	Helminthosporium sp.		
	Mucor mucedo		
	Sclerotium rolfsii	*Sclerotium rolfsii*	Root
	S. minor		
	Phoma lingam		
	P. glomerata		

Fungi isolated from soils of root vegetable fields

Name of vegetable fields	Fungi isolated
Bhidiura, Allahabad	*Alternaria alternata*
	A. raphani
	Botrytis cinerea
	Fusarium oxysporum
	F. solani
	F. roseum
	F. species
	F. species
Zapharabad (Jaunpur)	*Alternaria species*
	Botrytis cinerea
	Fusarium oxysporum
	F. species
	F. solani
	F. species
	F. equiseti
	Sclerotium species
	Sclerotium rolfsii
	Rhizoctonia species
Jahnaipur (Pratapgarh)	*Fusarium oxysporum*
	F. species
	F. solani
	F. roseum
	F. species
	Botrytis cinerea
	Sclerotium rolfsii
	Sclerotium species
Mirzamurad (Varanasi)	*Botrytis cinerea*
	Fusarium oxysporum
	F. species
	F. solani
	F. species
	F. semitectum
	F. equiseti

Kanwal (Sant Ravi Das Nager)	*Botrytis cinerea* *Fusarium species* *F. species* *F. solani* *F. species* *F. oxysporum* *Sclerotium rolfsii* *Sclerotium species* *Rhizoctonia species*

The isolation studies revealed that species of *Alternaria, Botrytis, Cercospora, Fusarium* and *Sclerotium*, were associated with seeds and infected parts of root vegetabls. In all seven genera of Fungi imperfecti, 3 genera Ascomycetes and 2 genera of Phycomycetes were isolated. A close relationship was observed in number and types of fungi isolated in all the cases.

Symptoms of the diseases were studies in situ. Regular visits were maid and careful observation were taken regarding initiation of disease development of blight, spot and root symptoms and ultimate fate of the infected crops.

Pathogenicity test was performed following the Koch's pastulates, and its was observed that a larger number of fungi isolated from root vegetables were pathogenic to their respective host plants causing following diseases :-

Name of root vegetables	Pathogenic fungi	Nature of diseases
Beet (*Beta vulgaris*)	*Cercospora beticola*	Leaf-spot
	Botrytis cinerea	Rot
	Fusarium oxysporum	Rot, wilt and seed-rots
	Sclerotium rolfsii	Rot
Carrot (*Daucus carota*)	*Alternaria dauci*	Blight and seed-rots
	A. radicina	Rot
	Botrytis cinerea	Rot
	Cercospora carotae	Leaf-spot
	Fusarium oxysporum	Rot, wilt and seed-rots
	Sclerotium rolfsii	Rot
Radish (*Raphanus sativus*)	*Alternaria raphani*	Blight
	Botrytis cinerea	Rot
	Fusarium oxysporum	Rot and seed-rots
	Sclerotium rolfsii	Rot
Turnip (*Brassica rapa*)	*Alternaria raphani*	Blight and seed-rots
	Botrytis cinerea	Rot
	Fusarium oxysporum	Rot and seed-rots
	Sclerotium rolfsii	Rot

Pathogenicity tests showed that a large number of Fungi isolated from infected plant parts and seeds were pathogenic to their respective host plants causing wilt, seed-rot, leaf-blights, leaf-spots and root-rots.

It has been observed at the study area around Allahabad that most of the soils are alkaline in nature having pH more then 12. The environmental factors play a crucial role and singnificantly effects to the measures adopted under chemical and biocontrol approach. It is therefore suggested to amend the pH of soil before going to take advantages of such measures.

Storage studies revealed that seed samples of root vegetables viz. *Beta vulgaris, Daucus carota, Raphanus sativus* and *Brassica rapa* stored upto 12 months were heavily associated with both storage and field fungi and their infestation, decreased upto 3 years of storage. Machine and hand threshed seeds of the above crops showed severe fungal contamination than hand threshed seeds. Seeds stored in the tin containers as well as in the sacks placed in wheat straw yielded lesser number of fungi than those stored in tin container, earthen pots or on floor. Seeds stored in mixture showed less infestation than seeds stored alone.

The effect of moisture contents in the seeds of *Beta vulgaris, Daucus carota, Raphanus sativus* and *Brassica rapa* on fungal association revealed that seeds of above crops were free from fungal association at a lower moisture content below 7.5%. A large number of seeds were associated with different types of fungi, when moisture contents of seeds were above 10.5%. At higher moisture percentage (12.5 - 13.5) heavy invasion of species of *Aspergillus* was observed.

The pathogens grow within a wide temperature range of 5°C to 40°C with maximum growth at 26°C - 28°C. The temperature range 25°C - 30°C was to be optimum for the growth and sporulation of the pathogen i.e., *Alternaria, Cercospora, Botrytis, Fusarium* and *Sclerotium species*. There was scanty growth at higher 35°C - 40°C and lower 5°C - 10°C range and no sporulation at 5°C.

It was measured that eight most frequently encountered fungi *Alternaria dauci, A. radicina, A raphani, Botrytis cinerea, Cercospora beticola, C. dauci, Fusarium oxysporum* and *Sclerotium rolfsii* were found to cause a slight readuction in yield of root vegetable crops under investigation.

The survival studies showed that the pathogens were remained viable in diseased plant debris, seeds and soil for about eight months (till the next sowing season of crops). Diseased plant debris, infested seeds and field soils were observed as the carrier of primary source of inoculum for the pathogens.

Fungicides viz. Agrosan GN, Captan Ceresan dry, Difolatan, Dithan M-45, Dithan Z-78, Vitavax, Benelate, Plantvax, Thiram and Tecto-40 in-vitro completely checked the growth of *Alternaria dauci, A. raphani, A. radicina, Botrytis cinerea, Cercospora beticola, C. carotae, Fusarium oxysporum* and *Sclerotium rolfsii*. Seeds of *Beta vulgaris, Daucus carota, Raphanus sativus* and

Brassica rapa when treated with 500, 750 and 1000 ppm of above effective fungicides cause a decrease in fungal association.

To save the germ and to prevent seed-borne diseases of *Beta valgaris, Daucus carota, Raphanus sativus* and *Brassica rapa* caused by *Alternaria dauci, A. raphani, A. radicina, Botrytis cinerea, Cercospora carotae, C. beticola, Fusarium oxysporum* and *Sclerotium rolfsii*, seeds of above plants should be treated with fungicides like Agrosan, Ceresan, Thiram and Vitavax at 1000 ppm. Ceresan was found to be more effective.

The leaf extracts of 5 medicinal plants out of 15, viz. *Ocimum sanctum, Azadirachta indica, Strychnos nux-vomica, Allium cepe* and *Allium sativum* were found effective in inhibiting spore germination of test fungi as well as to check fungal contaminations at all the concentrations.

Studies on high yielding varieties of above vegetable crops, resistant to seed borne fungi fiz., Alternaria radicina, A. dauci, A. raphani, Botrytis cinerea, Cercospora beticola, C. carotae, Fusarium oxysporum and Sclerotium rolfsii were revealed that Crimson globe and Bush-E (Beta vulgaris), M-29 and Selection-1 (Daucus carota), Arka nisant and Pusa chetaki (Raphanus sativus) and Pusa swarnima and Golden ball (Brassica rapa), were found to be resistant to above pathogens.

CHAPTER - 11

REFERENCES

REFERENCES

Agarwal, G.P., 1955 : *D. Phil. Thesis,* University of Allahabad.

Agrawal, A.K. & Singh, O.V., 1974 : *Indian Phytopath,* **27** : 240-241.

Alexander, M., 1977 : Introduction to soil microbiology, *2nd ed. John Wiley,* New York.

Alice D. and Roo, A.V.; 1987 : IRAM, **12** : 28.

Alice, D., 1984 : *M.Sc. Thesis,* TNAV Coimbatore.

Ames, A., 1915 : *Phytopathology,* **5** : 11-19.

Amodar, J.M. and Miller, M.E., 1976 : *Proc. Amer, Path. Soc.,* **3** : 262.

Anahosur, K.H., Fazalnoor, K. and Govindu, H.C., 1973 : *Mysore Journal of Agricultural Science,* **7 (4)** : 580-584.

Anderson, F., 1952 : *Plants, Man and Life,* Little Brown Boston.

Antique, M., Khan A. and Prakash, D., 1982 : *Indian Phytopath,* **35** : 717-718.

Armolic, N., Dickson, J.G., Dickson, A.D., 1956 : *Phytopath.,* **46** : 457-461.

Arora, S., 1987 : *D.Phil Thesis,* Allahabad University.

Asplin, F.D. & Carnaghan, R.B.A., 1961 : Vet. Res., 73 : 1215-1219.

Atkinson, R.G., 1950 : *Canad. Jour. Res., Sect.* C, **28** : 288-317.

Atlas De Gotuzzo, Esther, 1970 : *Revta Fac. Agron. Vet. Univ. B. Aires,* **18** : 7-16.

Ausemus, E.R., 1943 : *Bot. Rev.,* 9 : 207-260.

Backman, P.A. & Hammond, J.M., 1976 : Pl. Dis. Report., 60 : 1-3:

Bagcti, D.K., Matai, S. and Chandha, S., 1977 : *Indian J. Agron,* **22** : 190.

Becker, G., 1962 : *Hand buch der pflanzenzuchfung,* **6**: 23-78.

Bedi, K.S., 1956 : *Indian Phytopath,* **9** : 39-43.

Bennet, C.W., 1921 : *Mich. State Uni. Agri. Exp. Sta. Tech. Bull.,* **53** : 1-40.

Bhadraiah, B. and Ramarao, P., 1987 : *Indian Phytopath,* **40** : 401-404.

Bhargava, P.K. and Khare, M.N., 1988a : *Indian Phytopath.*, **41** : 195-198.

Bhargava, S.N. & Shukla, D.N., 1978 : Proc. Nat. Acad., Sci. Ind, 40 (B) : 81-82.

Bhargava, S.N. and Singh, A.P., 1974-75 : *Indian Phytopath.*, **27** : 613-619.

Bhargava, S.N., 1962 : *D. Phil. Thesis*, University of Allahabad.

Biften, R.H., 1905 : *J. Agr. Sci.*, **1** : 4-48.

Bilgrami, K.S., Prasad, T. and Sinha, R.K., 1979 : Today and Tomorrow's Printers and Publishers, New Delhi, pp. 125.

Blair, I.C., 1943 : *Ann. Appl. Bial*, **30** : 118-127.

Bockstahler, H.W., 1940 : *Proc. Amer. Soc. Sugar Beet Technol.*, **3** : 191-198. *Abs. in Rev. Appl. Mycol.*, **22** : 50.

Bolkman, H.A., Antoonia, R. deSilva, A.R., D.E. and Cupertino, F.P., 1976 : *Plant Disease Reporter*, **60** : 545-548.

Boosalis, M.G. and Mankau, R., 1965 : In Ecology of soil borne plant pathogens. K.F. Baker and W.C. Synder eds. pp. 374-389. Berkeley University of California Press.

Brooke, P.J. and White, E.P., 1966 : *Ann. Rev. Phytopath*, **4** : 171-194.

Brown, W., 1924 : *Ann. Bot.*, **38** : 401-404.

Burkill, I.H., 1935 : A. Dictionary of the Economic Plants of Malaya Peninsula, Crown Agents, London.

Burroughs Rose Mary and Sauer, D.B., 1971 : Phytopath; 61 : 767-772.

Byford, W.J., 1977 : *Indian Phytopath.*, **26 (1)** : 39-43.

Byrde, R.J.V., 1977 : *Sci. Hort.* **20** : 14-18.

Caldweel, R.M., 1966 : U.S. Dep. Agric. ARS Publ., 33-110. Washington, D.C.

Chakrovorti, B.P. and Anil Kumar, 1975 : Current Research, **4 (10)** : 172.

Chand, J.N. and Jatian, S.S., 1969 : *Jawaherlal Nehru Krisi Viswa Vidyalaya, Res. J.*, **3** : 132-133.

Chopra, R.N., 1933 : *Indigenous Drugs of India*, the Art Press Colcutta.

Chowdhury, S., 1944 : *Jour. Indian Bot. Soc.*, **23** : 91-106.

Christensen, C.M. and Dorworth, C.E., 1966 : Phytopath. 56 : 412-418.

Christensen, C.M. and Kaufmann, H.H., 1965 : Ann. Rev. Phytopath., 3 : 69-84.

Christensen, C.M. and Kaufmann, H.H., 1968 : Agric. Extension Service, Univ. of Minnesota, Etension Folder. 226, 4p.

Christensen, C.M. and Kaufmann, H.H., 1969 : University of Minnesota Press. Minneapolis. 7+153 pp.

Christensen, C.M. and Linko, 1963 : Cerea Chem., 40 : 129-137.

Christensen, C.M. and Lopez, F.L.C., 1963 : Proc. ISTA, 28 : 701-711.

Christensen, C.M. and Mirocha, C.J., 1976 : Phytopath., 66 : 204-205.

Christensen, C.M., 1955 : Cereal Chemistry, 32 : 507-518.

Christensen, C.M., 1957 : *Bot. Rev.*, **23** : 108-134.

Christensen, C.M., 1967 : *Phytopath.*, **57** : 622-624.

Christensen, C.M., 1972 : Pl. Dis. Reptr. 56 : 173-175.

Chupp, C., 1935 : *Phytopathology*, **25** : 269-274.

Chupp, C., 1953 : Cornell, Univ., Ithaca, New York.

Cocharane, V.W., 1958 : *Physiology of fungi*. John Wiley & Sons, Inc, New York.

Cooke, R.J. and Papedick, R.I., 1970 : *Plant and soil*, **32** : 131-145.

Cotton, R.T. and Gray, H.E., 1948 : Prevention of grain storage, F.A.O. Agricultural study No. 2 p. 35-37. Food and Agricultural of the United Nations. Vashington D.C.

Cotton, R.T.; Walkden, H.H.; Wite, G.D. and Wilbur, D.A., 1953 : Kansas experiment station Bulletin., 359.

Cunningham, H.S., 1928 : *Phytopathology*, **18** : 717-751.

Daftari, L.N. and Verma, O.P., 1973 : *Hindustan Antibiotics Bulletin*, **15 (3)** : 91-92.

Deena, E. and Basukachary, K.C., 1984 : *Indian Phytopath*, **37** : 151-153.

Dempsey, A.H. and Chandler, W.A., 1963 : *Pl. Dis. Reptr.*, **47** : 325-327.

Deverall, B.J., 1965 : In the fungi (eds. G.C. Ains-Worth and A.S. Sussman), Vol, 1, Acad. Press, New York, pp. 543-550.

Dharmvir, Raychaudhuri, S.F. and Gaur, A., 1972 : *Curr. Sci.*, **41** : 148-149.

Dhingra, O.D., Nicholson, J.F. and Sinclair, J.B., 1973 : *Pl. Dis. Reptr.*, **57** : 185-187.

Dhyani, A.P., Sati, M.C. and Khulbe, R.D., 1990 : *Indian Phytopath.*, **43 (1)** : 110-113.

Dicson, J.C., 1956 : Diseases of field crops. second edition. McGraw-Hill, New York, p. 517.

Diener, U.L. and Davis, N.E., 1967 : *J. Amer, Oil. Chem. Soc.*, **44** : 256-263.

Diener, U.L., 1960 : *Phytopath.*, **50** : 220-223.

Doran, W.L. and Guba, E.F., 1928 : *Mass. Agr. Expt. Sta. Bul.*, **245**.

Draglono, S., 1978 : *Forsking Forsok; Land Bruket*, **29** : 161-174.

Dutta, G.R. and Roy, A.K., 1987 : *Indian Phytopath.*, **40 (4)** : 520-524.

Dwivedi, R.S. and Tandon, R.N., 1975 : *Proc. Nat. Acad. Sci. India*, **45 (B) B** : 111-116.

Dwivedi, R.S. and Tandon, R.N., 1976 : *Acta Bolanica India*, **4 (2)** : 139-143.

Dwivedi, S.K., 1990 : Proceeding of the Indian Science Congress, Cochin.

Fields, R.W., King, T.H., 1962 : *Phytopath*, **52** : 336-339.

Flor, H.H., 1955 : *Phytopathology*, **30** : 172.

Forsberg, J.L., 1949 : *Phytopath.*, **30** : 172.

Garrett, S.D., 1965 : K.F. Barker and W.C. Synder (eds) University of California Press.

Garrette, A.D., 1936 : *Ann. App. Biol.*, **23** : 667-669.

Gattlieb, D., 1964 : *Endeavour*, **23** : 85-89.

Giffiths, D.A., Hodson, A.C. and Christensen, C.M., 1959 : *J. Econ. Ent.* **52** : 514-518.

Grover, R.K. and Bansal, R.D., 1970 : *Indian Phytopath.*, **23** : 654-660.

Grover, R.K. and Dutt, S., 1972 : *Ind. J. Mycol* and *Pl. Path.*, **2 (2)** : 98-102.

Groves, J.W. and Skolko, A.J., 1944 : Canada, *Jour. Res.* Sect. C., **22** : 217-234.

Gupta, R.C., Saxena, A. & Pandey, K.N., 1984 : Madras Agric. J., **71** : 474-475.

Gupta, R.P., Srivastava, P.K., Srivastava, V.K. and Pandey, U.P., 1984 : *Seed Research*, **12 (1)** : 98-100.

Gupta, V.K. and Chohan, J.S., 1970 : *Indian Phytopath.*, **23** : 622-625.

Hafiz, A., 1952 : *Phytopathology.*, **42** : 422-424.

Hansen, H.P., 1934 : Copenhagen, Denmark, Year Book, pp. 1-74.

Hare, W.W., 1965 : *Phytopath*, **55** : 1162-1167.

Harne, S.M. and Nema, K.G., 1969 : JNKV *Res. J.*, **3 (2)** : 130-131.

Haware, M.P. and Joshi, L.K., 1974 : *Indian Phytopath.*, **27** : 236-237.

Hawker, L.E., 1950 : Physiology of fungi, University of London Press Ltd., London, pp. 360.

Hellberg, A. and Holk, H., 1972 : *Acta. Agric. Sc. Scand.*, **22** : 1370149.

Henis, Y., Ghaffar, A. and Baker, R., 1978 : *Phytopathology*, **68** : 900-907.

Hoffmans, H., 1860 : *Jb. Wiss, Bot.*, **2** : 267-337.

Holmus, F.O., 1954 : *Advan. Virus. Res.* : **2** : 1-30.

Hooker, A.L. and Saxena, K.M.S., 1971 : *Ann. Rev. Gen.*, **5** : 407-424.

Hooker, A.L., 1967 : *Ann. Rev., Phytopath*, **5** : 163-182.

Hooker, W.J., 1944 : *Phytopathology*, **34** : 606-612.

Horsfall, J.G., Heuberger, J.W., Sharvell, E.G. and Hamilton, J.M., 1940 : *Phytopathology*, **30** : 545-563.

Hummel, B.C.W., Cuédent, L.S., Christensen, C.M. and Geddes, W.F., 1954 : *Cereal Chem.*, **31** : 143-150.

International Seed Testing Association, 1966 : *Proc. Int. Seed Test Ass.*, **31** : 1-152.

Janardhan, K.K; Ganguly, D; Baruah, J.B. and Rao, P.P., 1963 : *Curr. Sci.*, **32** : 226-227.

Jayaraman, P. and Kalyanasundaram, I., 1989 : *Indian Phytopath.*, **42** : 67-72.

Jhamaria, S.L., Sharma, K.P. and Gupta, R.B.L., 1975 : *Indian Journal of Mycology and Plant Pathology*, **5 (2)** : 212-213.

Jharia, H.K., Khare, M.N. and Amarchand, 1977 : *Indian Phytopath.*, **30** : 341-343.

Joffe, A.Z. and Palti J., 1972 : *Phytopath.* Z. **73** : 123-148.

Johnson, E.M. and Valleau, W.D., 1949 : *Ibid.*, **39** : 763-770.

Jones, L., Leeper, J.C. and Frederiksen, R.A., 1972 : *Phytopath.*, **62** : 817-819.

Joshi, R.N., 1971 : *Leaf protein : Its Agronomy. Preparation Quality and Use Ed. N.W. Pirie Black well Oxford.*

Kajansoon, P. and Mathur, R.S., 1961 : *Proc. Nat. Acad. Sci. India*, **31** : 416-421.

Kandian, O.P. and Suryanarayan, D., 1971 : Haryana *Agric. Univ. J. Res.*, **1** : 32-35.

Kapoor, A.; Mahor, R.; Vaishampayan, N. and Gautam, N., 1981 : *Antifungal spectrum of some petal extract geobios*, **8** : 86-87.

Karwasra, S.S. and Singh Mohinder, 1982 : *Indian Phytopath.*, **35** : 501-502.

Kennedy, B.W., 1964 : *Phytopath.*, **54** : 771-774.

Kenneth, H., Garren, B.B., Higgins, J.G. and Furtal, 1947 : *Phytopath.*, **37** : 669-679.

Khandelwal, G.L. and Prasada, R., 1970 : *Indian Phytopath.*, **23** : 37-43.

Kirtikar, K.R. and Basu, B.D., 1935 : *Indian medicinal plant Published by Lalit Mohan Basu, Allahabad.*

Krogh, R., Hosselager, E., Hald, B. and Kjoller, A., 1966 : *Royal vet. Agric. Coll.* Copenhagen Yearbook : **1966** : 129-147.

Kulik, M.M., 1973 : *Seed Sci. and Tech.* **1** : 805-810.

Kumar, J.C., Sharma, B.N., Sharma, P.B. and Paul, Y., 1974 : *Indian J. Hort*, **31** : 262-267.

Kumar, K. Jitendra Singh and Saksena, H.K., 1983 : *Indian Phytopath*, **36 (4)** : 716-718.

Kumar, R. and Kumar, S., 1980 : *Indian Phytopath.*, **33** : 620-621.

Lauritzen, J.I., 1926 : *Jour. Agr. Res.*, **33** : 1025-1041.

LeClerg, E.L., 1935 : *Ibid.*, **25** : 234-243.

Leelavathy, K.M., 1969 : *Pl. and Soil*, **XXX** : 473-476.

Leonard, O.A., 1940 : *Phytopathology*, **30** : 659-666.

Lichtenthaler, H.K., 1975 : *Plant Physiol.*, **34** : 357-358.

Lilly, V.G. and Barnett, H.L., 1951 : Physiology of Fungi McGraw Hill Book Company Inc., N.Y. pp. 463.

Link, K.P. and Walker, J.C., 1933 : *J. Biol. Chem.*, **100** : 379-383.

Lutey, R.W. and Christensen, C.M., 1963 : *Phytopathology*, **53** : 713-717.

Mahajan, P.D. and Mora, W.D., 1989 : *Indian Phytology*, **42** : 344.

Mali, J.B. and Jain, M.B., 1985 : *Curr. Res. Reptr.*, **1** : 8-10.

Massey, L.M., 1926 : *Phytopathology*, **16** : 509-523.

Mc. Donald, J.D. and Leach, L.D., 1976 : *Phytopath.*, **66 (7)** : 868-872.

McKay, M.B., and Pool, V.W., 1918 : *Ibid.*, **8** : 119-136.

McLean, D.M., 1960 : *J. Rio Grande Hort. Soc.*, **14** : 169-171.

Mehrotra, B.S., 1972 : All India seminar on a current trends in plant sciences. pp. 6-7.

Meiler, F.C., et al., 1922 : *Phytopatholgy*, **12** : 157-166.

Miller, P.M., 1972 : *Pl. Dis. Repter.*, **56 (7)** : 612-614.

Mohanty, A.K., Polai, D. and Mohanty, N.N., 1981 : *Indian J. Mycol. Pl. Pathol.*, **11** : 95-97.

Mukhopadhyay, A.N., 1969 : *Indian Phytopath.*, **22** : 135-138.

Muskett, A.E. and Malone, J.P., 1941 : *Ann. Appl. Biol.*, **28** : 8-13.

Narain, U., 1978 : *Ph.D., Thesis*, Kanpur University, Kanpur.

Naseema, A., Bakrishnan, S. and Nair, M.C., 1983 : *Agric. Res. J. of Kerala*, **21 (2)** : 32-37.

Neeragard, P., 1977 : *Seed Pathology*, Vol.-I and Vol.-II Mc. Millan Press Ltd. London Basingstoke, pp. 1187.

Nene, Y.L. and Srivastava, S.B.L., 1971 : *FAO Pl. Prot. Bull.*, **19** : 66-67.

Nene, Y.L. and Thapaliyal, P.N., 1979 : Fungicides in plant disease control Oxford and IBM Publishing Co., pp. 51.

Nene, Y.L., Agrawal, V.K. and Srivastava, S.S.L., 1969 : *Pesticides*, **3** : 26-27.

Nicholsan, J.F. and Sinclair, J.B., 1973 : *Pl. Dis. Reptr.*, **57** : 770-774.

Niles, E.V., 1976 : *Tropical Sciences*, **18** : 115-124.

Orton, W.A., 1990 : *Bull. Div. Veg. Physiol, Path.*, U.S. Deptt. of Agric. No. **27** : 26.

Pandey, R.R. and Dwivedi, R.S., 1987 : *Indian Phytopath.*, **40** : 248-250.

Pandey, R.S.; Bhargava, S.N.; Shukla, D.N. and Dwivedi, D.K., 1983 : *Revista nexinanade phytopathologia*, **2** : 15-16.

Papavizas and Christensen, C.M., 1958 : *Cereal Chem.*, **35** : 27-34.

Park, C.I. and Kim, W.G., 1986 : *Korean J. of Mycology*, **4 (1)** 89-91.

Patil, H.B. and Patil, A.A., 1986 : *South Indian Hort.*, **34** : 266-270.

Prabhu, A.S. and Prasada, 1967 : *Proc. Int. Seed test. Assoc.* **32** : 647-654.

Prabhu, A.S., 1965 : *Ph.D. Thesis*, I.A.R.I., New Delhi.

Prasad A. and Prasad, B.K., 1987 : *India J. of Mycol. and Pl. Path.* **17 (2)** : 191-192.

Prasad, P. and Reddy, M.N., 1986 : *Curr. Sci.*, **55** : 630.

Prasad, T. and Singh, B.K., 1983 : *Biological Bulletin of India*, **5 (2)** : 85-88 [En, 10 Ref. 1 Tab.] Bhagalpur University, Bhagalpur, India.

Qasem, S.A. and Christensen, C.M., 1958 : *Phytopathology*, **58** : 703-709.

Radhalkar, P.W. and Neergard, P., 1969 : *Hindustan Antibiotic. Bull.*, **II** : 163-165.

Rai, M., Singh, D.V., 1986 : *Pesticide*, **20 (12)** : 38-40. [En, 7 ref.] Dep. Pl., path. Chandra Shekhar Azad Univ. Agric and Tech., Kanpur, 208002 India.

Rajagopalan, B. & Wilson, K.J., 1972 : *Indian J. Microbiology*, **12** : 100-103.

Rajpurohit, T.S. and Prasad, N., 1982 : *Geobios*, **9** : 142-144.

Rangaswami, G., 1969 : *Tropical Agriculturist*, **CXXV** : 1-8.

Rangel, J.F., 1945 : *Phytopathology*, **35** : 1002-1007.

Raper, K.B. and Thom, C., 1944 : *Mycologia*, **36** : 555-575.

Rashmi, N. and Mehrotra, R.S., 1990 : *J. Ind. Bot. Soc.*, **69** : 43-46.

Richard, W. Fields and Thomas H. King, 1962 : *Phytopath.*, **52** : 336-339.

Riker, A.J. and Riker, R.S., 1936 : John Swift & Co. St. Louis, MO.

Roane, C.W., 1972 : *Ann. Rev. Phytopath.*, **11** : 463-486.

Rouatt, J.W. and Katznelson, H., 1961 : *J. App. Bacterial*, **24** : 164-171.

Saharan, G.S. and Gupta, V.K., 1974 : *Indian I. Myceal Plant Path.*, **3** : 222-224.

Saksena, R.K. and Mehrotra, B.S., 1952 : *Proc. Nat. Acad. Sci.*, India, **22B** : 22-43.

Sekhawat, P.S. and Prashad, R., 1971 : *Indian Phytopath.* **24** : 800-802.

Sen, Gupta, P.K. and Roy, S., 1971-72 : *Fupflkranklaund p. flan.* **78** : 670-674.

Shekhawat, P.S. and Prasada, R., 1971 : *Indian Phytopath.*, **24** : 800-802.

Shivpuri, A., Siradhana, B.S. and Bansal, R.K., 1988 : *Indian Phytopath.*, **41** : 444-446.

Shukla, B.N. and Singh, B.P., 1993 : *Indian Journal of Mycology and Plant Pathology*, **3 (2)** : 231-232.

Shukla, D.N., 2000 : *D.Sc. Thesis*, University of Allahabad.

Shukla, D.N., Singh, N.K. & Bhargava, S.N., 1978 : Stem blight of wheat caused by *Alternaria alternata. Curr. Sci.*, **47** : 142.

Shukla, R., 1987 : *Indian, J. Mycol. Pl. Pathol.*, **17** : 335-336.

Shuphan, W. and Weiller, H., 1967 : *Qual. Plant. Matter. Veg.*, **15** : 81-101.

Singh, A. and Bhowmik, T.P., 1985 : *Indian Phytopath.*, **38** : 35-38.

Singh, K.K. and Singh, Premlata, 1986 : *Indian Phytopath.*, **36** : 356-357.

Singh, M., 1887 : *Farm. Sci. J.*, **2** : 116-119.

Singh, P.N. and Singhal, Geeta, 1983 : *70th Annual Session of Indian Science Congress Ass. Section of Botany*, Tilrupati 3 to 8 Jan.

Singh, R.S. and Nene, Y.K., 1965 : *Pl. Dis. Reptr.*, **49** : 114-118.

Sinha, D.C. and Prasad, R.K., 1989 : *Indian Phytopath.*, **42 (1)** : 177-179.

Sinha, P.P. and Prasad, R.K., 1989 : *Indian J. Mycol. Pl. Pathol.*, **19** : 204-205.

Smedegaard-Petersen, V., 1974 : Royal Vet. *Aagric. Univ., Copenhagen Yearbook*, **1974** : 108-117.

Sohi, H.S. and Mahalay, M.N., 1974 : *Ind. J. Mycol. and Pl. Path.*, **4** : 25-28.

Spalding, D.H. and Reeder, W.F., 1972 : *Pl. Dis. Reptr.*, **56** : 751-753.

Srivastava, M.P. & Tandon, R.N., 1971 : *Indian Phytopath.*, **24** : 396-397.

Srivastava, R.C., 1979 : *Indian Phytopath.*, **32** : 289-290.

Srivastava, S., 1992 : *D. Phil. Thesis*, Allahabad University.

Standberg, J.O., Basset, M.J., Peterson, C.E. and Berger, R.D., 1972 : *Hort Science*, 7 : 345.

Stewart, D., 1931 : *Phytopathology*, **21** : 59-70.

Stover, R.H., 1953 : *J. Bot.*, **31** : 693-697.

Strider, D.L., 1962 : *Res. and Frng., N.C.*, **20** : 3-4.

Suryanarayana, D. and Bhombe, B., 1961 : *Indian Phytopath*, **14** : 30-41.

Swaminathan, R. and Sullia, S.B., 1969 : *Curr. Sci.*, **39** : 282-284.

Swaroop, J.L. and Mathur, R.S., 1972 : *Indian Phytopath.*, **15** : 125.

Tandon, R.N. and Bhargava, S.N., 1960 : *Proc. Nati. Acad., Sci.*, India, **30 B** : 257-265.

Tervet, I.W., 1945 : *Phytopathology*, **35** : 3-15.

Thomas, H.R., 1943 : *Phytopathology, 33* : 114-125.

Tiwari, D.K., 1986 : D. Phil. Thesis, Allahabad University.

Tripathi, N.N.; Dubey, N.K.; Tripathi, R.D. and Dixit, S.N., 1983 : *Indian phytopath.*, **36** : 286-287.

Tripathi, R.D.; Srivastava, H.S. and Dixit, S.N., 1978 : Fungitoxic Principle from the leavus of Lewsonia inermis Lam., Experientia. **34** : 51-52.

Tu, C.C. and Cheng, Y.H., 1976 : *Journal of Agricultural Research of China*, **25 (2)** : 134-140.

Tuite, J. and Christensen, C.M., 1955 : *Cereal Chemistry*, **32** : 1-11.

Tuite, J., 1959 : *Pl. Dis. Reptr.* **43** : 470.

Tuite, J., 1961 : *Pl. Dis. Reptr.* **45** : 212-215.

Ullstrup, A.J., 1961 : *U.S. Dep. Agric. and Purdue Univ. Agric. Exp. Sta. Agric.* Handbook No. 1991, 29p.

Ungaro, M.R.G. and Azevedo, J.L., 1984 : *Fitopathologia Brasileira*, **9** : 89-100.

Valaskova, E., 1962 : *Acta Pruhonic.* 61-71.

Vidyashekaran, P., Thulasidas, G. & Kandaswa, T.K., 1980 : *Indian Phytopath.*, **35 (2)** : 259-262.

Vishnupurikar, R.M. and Godbole, G.M., 1989 : *Indian Phytopath.*, **42** : 344.

Vishwakarma, S.N., 1990 : *Indian J. Mycol. Pl. Pathol,* **20** : 95-96.

Vyas, S.C. and Nene, Y.L., 1975 : JNKVV. *Res. Jaur.* **8 (1/2)** : 50-54.

Walker, J.C., 1952 : Disease of vegetable crops *McGraw Hill Book Co., Inc.*, New York.

Walker, J.C., 1965 : Disease resistance in the vegetables crops, Ill Bot. Rev. **31** : 331-380.

Wells, J.M., 1972 : *Phytopath.* **62** : 129-133.

Whittle, A.M., 1977 : *Trans-British Mycol. Soc.*, **69** : 47-57.

Wiant, J.S., 1941 : *U.S. Dept. Agr. Misc. Pub.*, **440**.

Wilson, J.D., 1944 : *Ohio Agr. Expt. Sta. Bimo. Bul.*, **29** : 63-73.

Wilson, J.D., 1962 : *Plant Dis. Reptr.*, **46** : 124-127.

Yadav, L.B., Prasad, Y. and Narain, U., 1989 : *Indian J. Pl. Pathol,* **7** : 72-73.

Zimer, D.E. and Zimmermann, D.C., 1972 : *Crop. Sci.*, **12** : 859-861.